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(57) Abstract

An enzyme capable of degrading arabinoxylan is described. In addition, there is described a nucleotide sequence coding for the same and a promoter for controlling the expression of the same.

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ASPERGILLUS ARABINOFURANOSIDASE

The present invention relates to an enzyme. In addition, the present invention relates to a nucleotide sequence coding for the enzyme. Also, the present invention relates to a promoter, wherein the promoter can be used to control the expression of the nucleotide sequence coding for the enzyme.

In particular, the enzyme of the present invention is an arabinofuranosidase enzyme having arabinoxylan degrading activity.

It is known that it is desirable to direct expression of a gene of interest ("GOI") in certain tissues of an organism - such as a filamentous fungus (such as *Aspergillus Niger*) or even a plant crop. The resultant protein or enzyme may be useful for the organism itself. For example, it may be desirable to produce crop protein products with an optimised amino acid composition and so increase the nutritive value of a crop. For example, the crop may be made more useful as a feed.

In the alternative, it may be desirable to isolate the resultant protein or enzyme and then use the protein or enzyme to prepare, for example, food compositions. In this regard, the resultant protein or enzyme can be a component of the food composition or it can be used to prepare food compositions, including altering the characteristics or appearance of food compositions. It may even be desirable to use the organism, such as a filamentous fungus or a crop plant, to express non-plant genes, such as for the same purposes.

Also, it may be desirable to use an organism, such as a filamentous fungus or a crop plant, to express mammalian genes. Examples of the latter products include interferons, insulin, blood factors and plasminogen activators. It is also desirable to use micro-organisms, such as filamentous fungi, to prepare products from GOIs by use of promoters that are active in the micro-organisms.

Fruit and vegetable cell walls largely consist of polysaccharide, the major components being pectin, cellulose and xyloglucan (R.R. Selvendran and J.A. Robertson, IFR Report 1989). Numerous cell wall models have been proposed which attempt to incorporate the essential properties of strength and flexibility (P. Albersheim, Sci. Am. 232, 81-95, 1975; P. Albersheim, Plant Biochem. 3rd Edition (Bonner and Varner), Ac. Press, 1976; T. Hayashi, Ann. Rev. Plant Physiol. & Plant Mol. Biol., 40, 139-168, 1989).

The composition of the plant cell wall is complex and variable. Polysaccharides are mainly found in the form of long chains of cellulose (the main structural component of the plant cell wall), hemicellulose (comprising various ß-xylan chains) and pectic substances (consisting of galacturonans and rhamnogalacturonans; arabinans; and galactans and arabinogalactans). From the standpoint of the food industry, the pectic substances, arabinans in particular, have become one of the most important constituents of plant cell walls (Whitaker, J.R. (1984) Enzyme Microb. Technol., 6,341).

One form of plant polysaccharide is arabinan. A review of arabinans may be found in EP-A-0506190. According to this document, arabinans consist of a main chain of α-(1→5) groups linked to one another. Side chains are linked α-(1→3) or sometimes α-(1→2) to the main α-(1→5)-L-arabinan backbone. In apple, for example, one third of the total arabinose is present in the side chains. The molecular weight of arabinan is normally about 15 kDa.

Arabinans are degraded by enzymes collectively called arabinases. In this regard, arabinan-degrading activity is the ability of an enzyme to release arabinose residues, either monomers or oligomers, from arabinan backbones or from arabinan-containing side chains of other hemicellulose backbone structures such as arabinogalactans, or even the release of arabinose monomers via the cleavage of the 1→6 linkage between the terminal arabinofuranosyl unit and the intermediate glucosyl unit of monoterpenyl α-L-arabinofuranosyl glucosides.

The activity of the arabinan degrading enzymes of EP-A-0506190 include: a) the ability to cleave (1→2)-α-L-arabinosidic linkages; b) the ability to cleave (1→3)-α-L-arabinosidic linkages; c) the ability to cleave (1→5)-α-L-arabinosidic linkages; d) the ability to cleave the 1→6 linkage between the terminal arabinofuranosyl unit and the intermediate glucosyl unit of monoterpenyl α-L-arabinofuranosyl glucosides.

Arabinan-degrading enzymes are known to be produced by a variety of plants and microorganisms, among these, fungi such as those of the genera *Aspergillus*, *Corticium*, *Rhodotorula* (Kaji, A. (1984) Adv. Carbohydr. Chem. Biochem., 42, 383), *Dichotomitus* (Brillouet et al. (1985) Carbohydrate Research, 144, 113), *Ascomycetes* and *Basidomycetes* (Sydow, G. (1977) DDR Patent Application No. 124,812).

Another plant polysaccharide is xylan, whose major monosaccharide unit is xylose. Xylans are abundant components of the hemicelluloses. In monocotyledonous plants the dominant hemicellulose is an arabinoxylan, in which arabinose side chains are attached to a backbone of xylose residues.

Arabinoxylans are carbohydrates found in the cell wall of cereals. A review of arabinoxylans and the enzymatic degradation thereof may be found in Voragen *et al* (1992 Characterisation of Cereal Arabinoxylans, Xylans and Xylanases pages 51-67, edited by J. Visser published by Elsevier Science Publishers).

Typically, arabinoxylans comprise a xylose backbone linked together via β-1,4- bonds. The xylose backbone is substituted with L-arabinose residues which are linked via α-1 bonds to the 2 or 3 position of the xylose residues. The xylose residues can be single or double substituted. In addition to substitution with arabinose the xylose residues can be substituted with acetyl groups, glucuronic acid and various other carbohydrates. The arabinose residues can be further substituted with phenolic acids such as ferulic acid and coumaric acid. The degree and kind of substitution depends on the source of the particular arabinoxylan.

Arabinoxylans are found in cereal cell wall where they are part of the secondary cell wall. Arabinoxylans form about 3 % of wheat flour - part of it is water soluble (WSP), part of it is water insoluble (WIP).

Despite the fact that the arabinoxylans amount to only about 3 % of wheat the importance of the arabinoxylan fraction is much higher. This is because the arabinoxylans of cereals act as hydrocolloids, as they form a gel like structure with water. For example, the arabinoxylans of wheat flour bind up to 30% of the water in a dough despite the fact that they amount to only 3 % of the dry matter. When arabinoxylans bind water they increase the viscosity of the ground cereals and to such an extent that the cereals can become difficult to manage.

The rheological properties of several systems where ground cereals are used can be manipulated using enzymes that degrade arabinoxylans. In modern bakery it is advantageous to reduce the viscosity of the dough in order to reduce the energy needed to process the doughs and also to get a higher volume of the bread. This is usually achieved by using enzymes that can degrade the xylose backbone of arabinoxylans.

Enzymes that only cleave the arabinose side chains from the xylan backbone of arabinoxylan are, for the purposes of this application, collectively called arabinoxylan degrading enzymes.

In feeds based on cereals, arabinoxylans in the cereals can increase the viscosity of the fluids in the intestines of the animals after the feeds have been ingested. This is a problem as it causes discomfort, such as indigestion, to the animals. Also, the nutritive value of the feeds is reduced. These problems can be avoided by addition of enzymes that degrade the arabinoxylan (such as xylanases) to the feed to avoid indigestion and to increase the nutritive value of the feed. However, some enzymes that degrade the arabinoxylans (especially some of the xylanases) require the presence of unsubstituted backbones and so their activity can be limited.

Further discussions on arabinoxylans can be found in Xylans and Xylanases (1992, edited by J. Visser published by Elsevier Science Publishers).

An arbinoxylan degrading enzyme is (1,4)-ß-D-arabinoxylan arabinofuranohydrolase (AXH), as described by Kormelink *et al* 1991 (Kormelink, F.J.M., Searle-Van Leeuwen M.J.F., Wood. T.M., Voragen, A.G.J.(1991) Purification and characterization of a (1,4)-*β*-D-arabinoxylan arabinofuranohydrolase from *Aspergillus awamori*. Appl. Microbiol. Biotechnol. 25:753-758). However, this document provides no sequence data for the enzyme or the nucleotide sequence coding for same or for the promoter for the same.

Clearly, it would be useful to be able to degrade arabinoxylans, preferably by use of recombinant DNA techniques.

The present invention seeks to provide an enzyme having arabinoxylan degrading activity; preferably wherein the enzyme can be prepared in certain or specific cells or tissues, such as in just a specific cell or tissue, of an organism, typically a filamentous fungus, preferably of the genus *Aspergillus*, such as *Aspergillus niger*, or even a plant.

Also, the present invention seeks to provide a GOI coding for the enzyme that can be expressed preferably in specific cells or tissues, such as in certain or specific cells or tissues, of an organism, typically a filamentous fungus, preferably of the genus *Aspergillus*, such as *Aspergillus niger*, or even a plant.

In addition, the present invention seeks to provide a promoter that is capable of directing expression of a GOI, such as a nucleotide sequence coding for the enzyme according to the present invention, preferably in certain specific cells or tissues, such as in just a specific cell or tissue, of an organism, typically a filamentous fungus, preferably of the genus *Aspergillus*, such as *Aspergillus niger*, or even a plant. Preferably, the promoter is used in *Aspergillus* wherein the product encoded by the GOI is excreted from the host organism into the surrounding medium.

Furthermore, the present invention seeks to provide constructs, vectors, plasmids, cells, tissues, organs and organisms comprising the GOI and/or the promoter, and methods of expressing the same, preferably in specific cells or tissues, such as expression in just a specific cell or tissue, of an organism, typically a filamentous fungus, preferably of the genus *Aspergillus*, or even a plant.

According to a first aspect of the present invention there is provided an enzyme obtainable from *Aspergillus*, wherein the enzyme has the following characteristics: a MW of 33,270 D ± 50 D; a pI value of about 3.7; arabinoxylan degrading activity; a pH optima of from about 2.5 to about 7.0 (more especially from about 3.3 to about 4.6, more especially about 4); a temperature optima of from about 40°C to about 60°C (more especially from about 45°C to about 55°C, more especially about 50°C); and wherein the enzyme is capable of cleaving arabinose from the xylose backbone of an arabinoxylan.

According to a second aspect of the present invention there is provided an enzyme having the sequence shown as SEQ. I.D. No. 1 or a variant, homologue or fragment thereof.

According to a third aspect of the present invention there is provided an enzyme coded by the nucleotide sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.

According to a fourth aspect of the present invention there is provided a nucleotide sequence coding for the enzyme according to the present invention.

According to a fifth aspect of the present invention there is provided a nucleotide sequence having the sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.

According to a sixth aspect of the present invention there is provided a promoter having the sequence shown as SEQ. I.D. No. 3 or a variant, homologue or fragment thereof or a sequence complementary thereto.

According to a seventh aspect of the present invention there is provided a terminator having the nucleotide sequence shown as SEQ. I.D. No. 13 or a variant, homologue or fragment thereof or a sequence complementary thereto.

According to an eighth aspect of the present invention there is provided a signal sequence having the nucleotide sequence shown as SEQ. I.D. No. 14 or a variant, homologue or fragment thereof or a sequence complementary thereto.

According to a ninth aspect of the present invention there is provided a process for expressing a GOI by use of a promoter, wherein the promoter is the promoter according to the present invention.

According to a tenth aspect of the present invention there is provided the use of an enzyme according to the present invention to degrade an arabinoxylan.

According to an eleventh aspect of the present invention there is provided a combination of enzymes to degrade an arabinoxylan, the combination comprising an enzyme according to the present invention and a xylanase.

According to a twelfth aspect of the present invention there is provided plasmid NCIMB 40703, or a nucleotide sequence obtainable therefrom for expressing an enzyme capable of degrading arabinoxylan or for controlling the expression thereof or for controlling the expression of another GOI.

According to a thirteenth aspect of the present invention there is provided a signal sequence having the sequence shown as SEQ. I.D. No. 15 or a variant, homologue or fragment thereof.

According to a fourteenth aspect of the present invention there is provided the use of the enzyme according to the present invention in the manufacture of a medicament or foodstuff to reduce or prevent indigestion and/or increase digestibility and/or increase nutrient absorption.

According to a fifteenth aspect of the present invention there is provided an arabinofuranosidase enzyme having arabinoxylan degrading activity, which is immunologically reactive with an antibody raised against a purified arabinofuranosidase enzyme having the sequence shown as SEQ. I.D. No. 1.

According to a sixteenth aspect of the present invention there is provided an arabinofuranosidase promoter wherein the promoter is inducible by an intermediate in xylose metabolism.

According to a seventeenth aspect of the present invention there is provided a process of reducing the viscosity of a branched substrate wherein the enzyme degrades the branches of the substrate but not the backbone of the substrate.

According to a further aspect of the present invention there is provided the use of the enzyme of the present invention as a viscosity modifier.

According to a further aspect of the present invention there is provided the use of the enzyme of the present invention to reduce the viscosity of pectin.

Other aspects of the present invention include constructs, vectors, plasmids, cells, tissues, organs and transgenic organisms comprising the aforementioned aspects of the present invention.

Other aspects of the present invention include methods of expressing or allowing expression or transforming any one of the nucleotide sequence, the construct, the plasmid, the vector, the cell, the tissue, the organ or the organism, as well as the products thereof.

Additional aspects of the present invention include uses of the promoter for expressing GOIs in culture media such as a broth or in a transgenic organism.

Further aspects of the present invention include uses of the enzyme for preparing or treating foodstuffs, including animal feed.

Preferably the enzyme is coded by the nucleotide sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.

Preferably the nucleotide sequence has the sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.

Preferably the nucleotide sequence is operatively linked to a promoter.

Preferably the promoter comprises the sequence CCAAT.

Preferably the promoter is the promoter having the sequence shown as SEQ. I.D. No. 3 or a variant, homologue or fragment thereof or a sequence complementary thereto.

Preferably, the promoter comprises the 100 bps sequence from the *Xma* 111 to the *BamH*1 sites.

Preferably the promoter of the present invention is operatively linked to a GOI.

Preferably the GOI comprises a nucleotide sequence according to the present invention.

Preferably the transgenic organism is a fungus.

Preferably the transgenic organism is a filamentous fungus, more preferably of the genus *Aspergillus*.

Preferably the transgenic organism is a plant.

Preferably, in the use, the enzyme is used in combination with a xylanase, preferably an endoxylanase.

Highly preferred embodiments of each of the aspects of the present invention do not include any one of the native enzyme, the native promoter or the native nucleotide sequence in its natural environment.

Preferably, in any one of the plasmid, the vector such as an expression vector or a transformation vector, the cell, the tissue, the organ, the organism or the transgenic organism, the promoter is present in combination with at least one GOI.

Preferably the promoter and the GOI are stably incorporated within the transgenic organism's genome.

Preferably the transgenic organism is a filamentous fungus, preferably of the genus *Aspergillus*, more preferably *Aspergillus niger*. The transgenic organism can even be a plant, such as a monocot or dicot plant.

A highly preferred embodiment is an enzyme obtainable from *Aspergillus*, wherein the enzyme has the following characteristics: a MW of 33,270 D ± 50 D; a pI value of about 3.7; arabinoxylan degrading activity; a pH optima of from about 2.5 to about 7.0 (more especially from about 3.3 to about 4.6, more especially about 4); a temperature optima of from about 40°C to about 60°C (more especially from about 45°C to about 55°C, more especially about 50°C); and wherein the enzyme is capable of cleaving arabinose from the xylose backbone of an arabinoxylan; wherein the enzyme has the sequence shown as SEQ. I.D. No. 1 or a variant, homologue or fragment thereof.

Another highly preferred embodiment is an enzyme obtainable from *Aspergillus*, wherein the enzyme has the following characteristics: a MW of 33,270 D ± 50 D; a pI value of about 3.7; arabinoxylan degrading activity; a pH optima of from about 2.5 to about 7.0 (more especially from about 3.3 to about 4.6, more especially about 4); a temperature optima of from about 40°C to about 60°C (more especially from about 45°C to about 55°C, more especially about 50°C); and wherein the enzyme is capable of cleaving arabinose from the xylose backbone of an arabinoxylan; wherein the enzyme is coded by the nucleotide sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment

thereof or a sequence complementary thereto.

The advantages of the present invention are that it provides a means for preparing an arabinofuranosidase enzyme having arabinoxylan degrading activity and the nucleotide sequence coding for the same. In addition, it provides a promoter that can control the expression of that, or another, nucleotide sequence.

Other advantages are that the enzyme of the present invention can affect the viscosity of ground cereals, such as dough, to ease the handling thereof and for example to get a higher volume of the bread.

The enzyme of the present invention is also advantageous for feed because it degrades arabinoxylan and thus increases the nutritive value of the feed. In addition, it reduces the viscosity of the arabinoxylan in the intestine of the animals and so reduces or prevents indigestion.

The combination of the use of the enzyme of the present invention with a xylanase is particularly advantageous because the enzyme of the present invention and the xylanase have a surprising and unexpected synergistic effect with each other.

In this regard, the enzyme of the present invention increases the degradative effect of the xylanase, and the xylanase increases the degradative effect of the enzyme of the present invention. It is believed that the activity of the xylanase is increased because the enzyme of the present invention provides a polysaccharide substrate having fewer substituted groups.

The present invention therefore provides an enzyme having arabinoxylan degrading activity wherein the enzyme can be prepared in certain or specific cells or tissues, such as in just a specific cell or tissue, of an organism, typically a filamentous fungus, preferably of the genus *Aspergillus*, such as *Aspergillus niger*. The enzyme may even be prepared in a plant.

More in particular, the enzyme of the present invention is capable of specifically cleaving arabinose from the xylose backbone of arabinoxylan.

The arabinofuranosidase of the present invention is different from the arabinofuranosidases previously known. In this regard, the previous described arabinofuranosidases - such as those of EP-A-0506190 - are characterised by their ability to degrade unbranched arabinan, and are assayed using p-nitrophenyl-arabinoside.

The arabinofuranosidase of the present invention does not degrade unbranched arabinan, and only a minor activity is seen on nitrophenyl-arabinoside. In contrast, the arabinofuranosidase of the present invention is useful for degrading arabinoxylan. Therefore, the arabinofuranosidase of the present invention is quite different from the previous isolated arabinofuranosidases.

Also, the present invention provides a GOI coding for the enzyme that can be expressed preferably in specific cells or tissues, such as in certain or specific cells or tissues, of an organism, typically a filamentous fungus, preferably of the genus *Aspergillus*, such as *Aspergillus niger*. The GOI may even be expressed in a plant.

In addition, the present invention provides a promoter that is capable of directing expression of a GOI, such as a nucleotide sequence coding for the enzyme according to the present invention, preferably in certain specific cells or tissues, such as in just a specific cell or tissue, of an organism, typically a filamentous fungus, preferably of the genus *Aspergillus*, such as *Aspergillus niger*, or even a plant. Preferably, the promoter is used in *Aspergillus* wherein the product encoded by the GOI is excreted from the host organism into the surrounding medium. The promoter may even be tailored (if necessary) to express a GOI in a plant.

The present invention also provides constructs, vectors, plasmids, cells, tissues, organs and organisms comprising the GOI and/or the promoter, and methods of expressing the same, preferably in specific cells or tissues, such as expression in just a specific cell or tissue, of an organism, typically a filamentous fungus, preferably of the genus

Aspergillus, or even a plant.

The terms "variant", "homologue" or "fragment" in relation to the enzyme include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acid from or to the sequence providing the resultant amino acid sequence has arabinoxylan degrading activity, preferably having at least the same activity of the enzyme shown in the sequence listings (SEQ I.D. No. 1 or 12). In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant enzyme has arabinoxylan degrading activity. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to SEQ ID NO. 1 shown in the attached sequence listings. More preferably there is at least 95%, more preferably at least 98%, homology to SEQ ID NO. 1 shown in the attached sequence listings.

The terms "variant", "homologue" or "fragment" in relation to the nucleotide sequence coding for the enzyme include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence codes for an enzyme having arabinoxylan degrading activity, preferably having at least the same activity of the enzyme shown in the sequence listings (SEQ I.D. No. 2 or 12). In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant nucleotide sequence codes for an enzyme having arabinoxylan degrading activity. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to SEQ ID NO. 2 shown in the attached sequence listings. More preferably there is at least 95%, more preferably at least 98%, homology to SEQ ID NO. 2 shown in the attached sequence listings.

The terms "variant", "homologue" or "fragment" in relation to the promoter include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence has the ability to act as a promoter in an expression system - such as the transformed cell or the transgenic organism according to the present invention. In

particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant nucleotide sequence has the ability to act as a promoter. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to SEQ ID NO. 3 shown in the attached sequence listings. More preferably there is at least 95%, more preferably at least 98%, homology to SEQ ID NO. 3 shown in the attached sequence listings.

The terms "variant", "homologue" or "fragment" in relation to the terminator or signal nucleotide sequences include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence has the ability to act as a terminator or codes for an amino acid sequence that has the ability to act as a signal sequence respectively in an expression system - such as the transformed cell or the transgenic organism according to the present invention. In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant nucleotide sequence has the ability to act as or code for a terminator or signal respectively. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to SEQ ID NO.s 13 and 14 (respectively) shown in the attached sequence listings. More preferably there is at least 95%, more preferably at least 98%, homology to SEQ ID NO.s 13 and 14 (respectively) shown in the attached sequence listings.

The terms "variant", "homologue" or "fragment" in relation to the signal amino acid sequence include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acid from or to the sequence providing the resultant sequence has the ability to act as a signal sequence in an expression system - such as the transformed cell or the transgenic organism according to the present invention. In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant nucleotide sequence has the ability to act as or code for a signal respectively. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to SEQ ID NO 15 shown in the attached sequence listings. More preferably there is at least

95%, more preferably at least 98%, homology to SEQ ID NO 15 shown in the attached sequence listings.

The above terms are synonymous with allelic variations of the sequences.

The term "complementary" means that the present invention also covers nucleotide sequences that can hybridise to the nucleotide sequences of the coding sequence or the promoter sequence, respectively.

The term "nucleotide" in relation to the present invention includes genomic DNA, cDNA, synthetic DNA, and RNA. Preferably it means DNA, more preferably cDNA for the coding sequence of the present invention.

The term "construct" - which is synonymous with terms such as "conjugate", "cassette" and "hybrid" - includes a GOI directly or indirectly attached to a promoter. An example of an indirect attachment is the provision of a suitable spacer group such as an intron sequence, such as the *Sh1*-intron or the ADH intron, intermediate the promoter and the GOI. The same is true for the term "fused" in relation to the present invention which includes direct or indirect attachment. In each case, it is highly preferred that the terms do not cover the natural combination of the gene coding for the enzyme ordinarily associated with the wild type gene promoter and when they are both in their natural environment. A highly preferred embodiment is the or a GOI being operatively linked to a or the promoter.

The construct may even contain or express a marker which allows for the selection of the genetic construct in, for example, a filamentous fungus, preferably of the genus *Aspergillus*, such as *Aspergillus niger*, or plants, preferably cereals, such as maize, rice, barley etc., into which it has been transferred. Various markers exist which may be used, such as for example those encoding mannose-6-phosphate isomerase (especially for plants) or those markers that provide for antibiotic resistance - e.g. resistance to G418, hygromycin, bleomycin, kanamycin and gentamycin.

The term "vector" includes expression vectors and transformation vectors.

The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression.

The term "transformation vector" means a construct capable of being transferred from one species to another - such as from an *E.coli* plasmid to a filamentous fungus, preferably of the genus *Aspergillus*. It may even be a construct capable of being transferred from an *E.coli* plasmid to an *Agrobacterium* to a plant.

The term "tissue" includes tissue *per se* and organ.

The term "organism" in relation to the present invention includes any organism that could comprise the promoter according to the present invention and/or the nucleotide sequence coding for the enzyme according to the present invention and/or products obtained therefrom, wherein the promoter can allow expression of a GOI and/or wherein the nucleotide sequence according to the present invention can be expressed when present in the organism.

Preferably the organism is a filamentous fungus, preferably of the genus *Aspergillus*, more preferably *Aspergillus niger*.

The term "transgenic organism" in relation to the present invention includes any organism that comprises the promoter according to the present invention and/or the nucleotide sequence coding for the enzyme according to the present invention and/or products obtained therefrom, wherein the promoter can allow expression of a GOI and/or wherein the nucleotide sequence according to the present invention can be expressed within the organism. Preferably the promoter and/or the nucleotide sequence is (are) incorporated in the genome of the organism.

Preferably the transgenic organism is a filamentous fungus, preferably of the genus *Aspergillus*, more preferably *Aspergillus niger*.

Therefore, the transgenic organism of the present invention includes an organism comprising any one of, or combinations of, the promoter according to the present invention, the nucleotide sequence coding for the enzyme according to the present invention, constructs according to the present invention, vectors according to the present invention, plasmids according to the present invention, cells according to the present invention, tissues according to the present invention or the products thereof. For example the transgenic organism can comprise a GOI, preferably an exogenous nucleotide sequence, under the control of the promoter according to the present invention. The transgenic organism can also comprise the nucleotide sequence coding for the enzyme of the present invention under the control of a promoter, which may be the promoter according to the present invention.

In a highly preferred embodiment, the transgenic organism does not comprise the combination of the promoter according to the present invention and the nucleotide sequence coding for the enzyme according to the present invention, wherein both the promoter and the nucleotide sequence are native to that organism and are in their natural environment. Thus, in these highly preferred embodiments, the present invention does not cover the native nucleotide coding sequence according to the present invention in its natural environment when it is under the control of its native promoter which is also in its natural environment. In addition, in this highly preferred embodiment, the present invention does not cover the native enzyme according to the present invention when it is in its natural environment and when it has been expressed by its native nucleotide coding sequence which is also in its natural environment and when that nucleotide sequence is under the control of its native promoter which is also in its natural environment.

The term "promoter" is used in the normal sense of the art, e.g. an RNA polymerase binding site in the Jacob-Mond theory of gene expression.

In one aspect, the promoter of the present invention is capable of expressing a GOI, which can be the nucleotide sequence coding for the enzyme of the present invention.

In another aspect, the nucleotide sequence according to the present invention is under the control of a promoter that allows expression of the nucleotide sequence. In this regard, the promoter need not necessarily be the same promoter as that of the present invention. In this aspect, the promoter may be a cell or tissue specific promoter. If, for example, the organism is a plant then the promoter can be one that affects expression of the nucleotide sequence in any one or more of stem, sprout, root and leaf tissues.

By way of example, the promoter for the nucleotide sequence of the present invention can be the α-Amy 1 promoter (otherwise known as the Amy 1 promoter, the Amy 637 promoter or the α-Amy 637 promoter) as described in our co-pending UK patent application No. 9421292.5 filed 21 October 1994. That promoter comprises the sequence shown in Figure 1.

Alternatively, the promoter for the nucleotide sequence of the present invention can be the α-Amy 3 promoter (otherwise known as the Amy 3 promoter, the Amy 351 promoter or the α-Amy 351 promoter) as described in our co-pending UK patent application No. 9421286.7 filed 21 October 1994. That promoter comprises the sequence shown in Figure 2.

Preferably, the promoter is the promoter of the present invention.

In addition to the nucleotide sequences described above, the promoters, particularly that of the present invention, could additionally include features to ensure or to increase expression in a suitable host. For example, the features can be conserved regions such as a Pribnow Box or a TATA box. The promoters may even contain other sequences to affect (such as to maintain, enhance, decrease) the levels of expression of the GOI. For example, suitable other sequences include the *Sh1*-intron or an ADH intron. Other sequences include inducible elements - such as temperature, chemical, light or stress inducible elements.

Also, suitable elements to enhance transcription or translation may be present. An example of the latter element is the TMV 5' signal sequence (see Sleat Gene 217 [1987]

217-225; and Dawson Plant Mol. Biol. 23 [1993] 97).

In addition the present invention also encompasses combinations of promoters and/or nucleotide sequences coding for proteins or enzymes and/or elements. For example, the present invention encompasses the combination of a promoter according to the present invention operatively linked to a GOI, which could be a nucleotide sequence according to the present invention, and another promoter such as a tissue specific promoter operatively linked to the same or a different GOI.

The present invention also encompasses the use of promoters to express a nucleotide sequence coding for the enzyme according to the present invention, wherein a part of the promoter is inactivated but wherein the promoter can still function as a promoter. Partial inactivation of a promoter in some instances is advantageous.

In particular, with the Amy 351 promoter mentioned earlier it is possible to inactivate a part of it so that the partially inactivated promoter expresses GOIs in a more specific manner such as in just one specific tissue type or organ.

The term "inactivated" means partial inactivation in the sense that the expression pattern of the promoter is modified but wherein the partially inactivated promoter still functions as a promoter. However, as mentioned above, the modified promoter is capable of expressing a GOI in at least one (but not all) specific tissue of the original promoter. One such promoter is the Amy 351 promoter described above.

Examples of partial inactivation include altering the folding pattern of the promoter sequence, or binding species to parts of the nucleotide sequence, so that a part of the nucleotide sequence is not recognised by, for example, RNA polymerase. Another, and preferable, way of partially inactivating the promoter is to truncate it to form fragments thereof. Another way would be to mutate at least a part of the sequence so that the RNA polymerase can not bind to that part or another part.

Another modification is to mutate the binding sites for regulatory proteins for example the CreA protein known from filamentous fungi to exert carbon catabolite repression, and thus abolish the catabolite repression of the native promoter.

The term "GOI" with reference to the present invention means any gene of interest. A GOI can be any nucleotide that is either foreign or natural to the organism (e.g. filamentous fungus, preferably of the genus *Aspergillus*, or a plant) in question. Typical examples of a GOI include genes encoding for proteins and enzymes that modify metabolic and catabolic processes. The GOI may code for an agent for introducing or increasing pathogen resistance. The GOI may even be an antisense construct for modifying the expression of natural transcripts present in the relevant tissues. The GOI may even code for a non-natural protein of a filamentous fungus, preferably of the genus *Aspergillus*, or a compound that is of benefit to animals or humans.

For example, the GOI could code for a pharmaceutically active protein or enzyme such as any one of the therapeutic compounds insulin, interferon, human serum albumin, human growth factor and blood clotting factors. In this regard, the transformed cell or organism could prepare acceptable quantities of the desired compound which would be easily retrievable from, the cell or organism. The GOI may even be a protein giving nutritional value to a food or crop. Typical examples include plant proteins that can inhibit the formation of anti-nutritive factors and plant proteins that have a more desirable amino acid composition (e.g. a higher lysine content than a non-transgenic plant). The GOI may even code for an enzyme that can be used in food processing such as chymosin, thaumatin and α-galactosidase. The GOI can be a gene encoding for any one of a pest toxin, an antisense transcript such as that for patatin or α-amylase, ADP-glucose pyrophosphorylase (e.g. see EP-A-0455316), a protease antisense or a glucanase.

The GOI can be the nucleotide sequence coding for the α-amylase enzyme which is the subject of our co-pending UK patent application 9413439.2 filed on 4 July 1994, the sequence of which is shown in Figure 3. The GOI can be the nucleotide sequence coding for the α-amylase enzyme which is the subject of our co-pending UK patent application 9421290.9 filed on 21 October 1994, the sequence of which is shown in Figure 4. The

GOI can be any of the nucleotide sequences coding for the ADP-glucose pyrophosphorylase enzymes which are the subject of our co-pending PCT patent application PCT/EP94/01082 filed 7 April 1994, the sequences of which are shown in Figures 5 and 6. The GOI can be any of the nucleotide sequences coding for the α-glucan lyase enzyme which are described in our co-pending PCT patent application PCT/EP94/03397 filed 15 October 1994, the sequences of which are shown in Figures 7-10.

In one preferred embodiment, the GOI is a nucleotide sequence coding for the enzyme according to the present invention.

As mentioned above, a preferred host organism is of the genus *Aspergillus*, such as *Aspergillus niger*. The transgenic *Aspergillus* according to the present invention can be prepared by following the teachings of Rambosek,J. and Leach,J. 1987 (Recombinant DNA in filamentous fungi: Progress and Prospects. CRC Crit. Rev. Biotechnol. 6:357-393), Davis R.W. 1994 (Heterologous gene expression and protein secretion in *Aspergillus*. In: Martinelli S.D., Kinghorn J.R.(Editors) *Aspergillus*: 50 years on. Progress in industrial microbiology vol 29. Elsevier Amsterdam 1994. pp 525-560), Ballance,D.J. 1991 (Transformation systems for Filamentous Fungi and an Overview of Fungal Gene structure. In :Leong,S.A., Berka R.M. (Editors) Molecular Industrial Mycology. Systems and Applications for Filamentous Fungi. Marcel Dekker Inc. New York 1991. pp 1-29) and Turner G. 1994 (Vectors for genetic manipulation. In: Martinelli S.D., Kinghorn J.R.(Editors) *Aspergillus*: 50 years on. Progress in industrial microbiology vol 29. Elsevier Amsterdam 1994. pp. 641-666). However, the following commentary provides a summary of those teachings for producing transgenic *Aspergillus* according to the present invention.

Filamentous fungi have during almost a century been widely used in industry for production of organic compounds and enzymes. Traditional japanese koji and soy fermentations have used *Aspergillus sp* for hundreds of years. In this century *Aspergillus niger* has been used for production of organic acids particular citric acid and for production of various enzymes for use in industry.

There are two major reasons for that filamentous fungi have been so widely used in industry. First filamentous fungi can produce high amounts of extracellular products, for example enzymes and organic compounds such as antibiotics or organic acids. Second filamentous fungi can grow on low cost substrates such as grains, bran, beet pulp etc. The same reasons have made filamentous fungi attractive organisms as hosts for heterologous expression according to the present invention.

In order to prepare the transgenic *Aspergillus*, expression constructs are prepared by inserting a GOI (such as an amylase or SEQ. I.D. No. 2) into a construct designed for expression in filamentous fungi.

Several types of constructs used for heterologous expression have been developed. The constructs contain the promoter according to the present invention (or if desired another promoter if the GOI codes for the enzyme according to the present invention) which is active in fungi. Examples of promoters other than that of the present invention include a fungal promoter for a highly expressed extracellular enzyme, such as the glucoamylase promoter or the α-amylase promoter. The GOI can be fused to a signal sequence (such as that of the present invention or another suitable sequence) which directs the protein encoded by the GOI to be secreted. Usually a signal sequence of fungal origin is used, such as that of the present invention. A terminator active in fungi ends the expression system, such as that of the present invention.

Another type of expression system has been developed in fungi where the GOI is fused to a smaller or a larger part of a fungal gene encoding a stable protein. This can stabilize the protein encoded by the GOI. In such a system a cleavage site, recognized by a specific protease, can be introduced between the fungal protein and the protein encoded by the GOI, so the produced fusion protein can be cleaved at this position by the specific protease thus liberating the protein encoded by the GOI ("POI"). By way of example, one can introduce a site which is recognized by a KEX-2 like peptidase found in at least some *Aspergilli*. Such a fusion leads to cleavage *in vivo* resulting in protection of the POI and production of POI and not a larger fusion protein.

Heterologous expression in *Aspergillus* has been reported for several genes coding for bacterial, fungal, vertebrate and plant proteins. The proteins can be deposited intracellularly if the GOI is not fused to a signal sequence. Such proteins will accumulate in the cytoplasm and will usually not be glycosylated which can be an advantage for some bacterial proteins. If the GOI is equipped with a signal sequence the protein will accumulate extracellulary.

With regard to product stability and host strain modifications, some heterologous proteins are not very stable when they are secreted into the culture fluid of fungi. Most fungi produce several extracellular proteases which degrade heterologous proteins. To avoid this problem special fungal strains with reduced protease production have been used as host for heterologous production.

For the transformation of filamentous fungi, several transformation protocols have been developed for many filamentous fungi (Ballance 1991, *ibid*). Many of them are based on preparation of protoplasts and introduction of DNA into the protoplasts using PEG and Ca^{2+} ions. The transformed protoplasts then regenerate and the transformed fungi are selected using various selective markers. Among the markers used for transformation are a number of auxotrophic markers such as *argB*, *trpC*, *niaD* and *pyrG*, antibiotic resistance markers such as benomyl resistance, hygromycin resistance and phleomycin resistance. A very common used transformation marker is the *amdS* gene of *A. nidulans* which in high copy number allows the fungus to grow with acrylamide as the sole nitrogen source.

Even though the enzyme, the nucleotide sequence coding for same and the promoter of the present invention are not disclosed in EP-B-0470145 and CA-A-2006454, those two documents do provide some useful background commentary on the types of techniques that may be employed to prepare transgenic plants according to the present invention. Some of these background teachings are now included in the following commentary.

The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted

genetic material.

Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27).

Thus, in one aspect, the present invention relates to a vector system which carries a promoter or nucleotide sequence or construct according to the present invention and which is capable of introducing the promoter or nucleotide sequence or construct into the genome of an organism, such as a plant.

The vector system may comprise one vector, but it can comprise two vectors. In the case of two vectors, the vector system is normally referred to as a binary vector system. Binary vector systems are described in further detail in Gynheung An et al. (1980), Binary Vectors, *Plant Molecular Biology Manual A3*, 1-19.

One extensively employed system for transformation of plant cells with a given promoter or nucleotide sequence or construct is based on the use of a Ti plasmid from *Agrobacterium tumefaciens* or a Ri plasmid from *Agrobacterium rhizogenes* An et al. (1986), *Plant Physiol.* 81, 301-305 and Butcher D.N. et al. (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208.

Several different Ti and Ri plasmids have been constructed which are suitable for the construction of the plant or plant cell constructs described above. A non-limiting example of such a Ti plasmid is pGV3850.

The promoter or nucleotide sequence or construct of the present invention should preferably be inserted into the Ti-plasmid between the terminal sequences of the T-DNA or adjacent a T-DNA sequence so as to avoid disruption of the sequences immediately surrounding the T-DNA borders, as at least one of these regions appear to be essential

for insertion of modified T-DNA into the plant genome.

As will be understood from the above explanation, if the organism is a plant, then the vector system of the present invention is preferably one which contains the sequences necessary to infect the plant (e.g. the *vir* region) and at least one border part of a T-DNA sequence, the border part being located on the same vector as the genetic construct.

Furthermore, the vector system is preferably an *Agrobacterium tumefaciens* Ti-plasmid or an *Agrobacterium rhizogenes* Ri-plasmid or a derivative thereof, as these plasmids are well-known and widely employed in the construction of transgenic plants, many vector systems exist which are based on these plasmids or derivatives thereof.

In the construction of a transgenic plant the promoter or nucleotide sequence or construct of the present invention may be first constructed in a microorganism in which the vector can replicate and which is easy to manipulate before insertion into the plant. An example of a useful microorganism is *E. coli*, but other microorganisms having the above properties may be used. When a vector of a vector system as defined above has been constructed in *E. coli*, it is transferred, if necessary, into a suitable *Agrobacterium* strain, e.g. *Agrobacterium tumefaciens*. The Ti-plasmid harbouring the promoter or nucleotide sequence or construct of the invention is thus preferably transferred into a suitable *Agrobacterium* strain, e.g. *A. tumefaciens*, so as to obtain an *Agrobacterium* cell harbouring the promoter or nucleotide sequence or construct of the invention, which DNA is subsequently transferred into the plant cell to be modified.

As reported in CA-A-2006454, a large amount of cloning vectors are available which contain a replication system in *E. coli* and a marker which allows a selection of the transformed cells. The vectors contain for example pBR 322, pUC series, M13 mp series, pACYC 184 etc.

In this way, the nucleotide or construct or promoter of the present invention can be introduced into a suitable restriction position in the vector. The contained plasmid is used for the transformation in *E.coli*. The *E.coli* cells are cultivated in a suitable nutrient

medium and then harvested and lysed. The plasmid is then recovered. As a method of analysis there is generally used sequence analysis, restriction analysis, electrophoresis and further biochemical-molecular biological methods. After each manipulation, the used DNA sequence can be restricted and connected with the next DNA sequence. Each sequence can be cloned in the same or different plasmid.

After each introduction method of the desired promoter or construct or nucleotide sequence according to the present invention in the plants the presence and/or insertion of further DNA sequences may be necessary. If, for example, for the transformation the Ti- or Ri-plasmid of the plant cells is used, at least the right boundary and often however the right and the left boundary of the Ti- and Ri-plasmid T-DNA, as flanking areas of the introduced genes, can be connected. The use of T-DNA for the transformation of plant cells has been intensively studied and is described in EP-A-120516; Hoekema, in: The Binary Plant Vector System Offset-drukkerij Kanters B.B., Alblasserdam, 1985, Chapter V; Fraley, et al., Crit. Rev. Plant Sci., 4:1-46; and An et al., EMBO J. (1985) 4:277-284.

Direct infection of plant tissues by *Agrobacterium* is a simple technique which has been widely employed and which is described in Butcher D.N. et al. (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208. For further teachings on this topic see Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27). With this technique, infection of a plant may be done on a certain part or tissue of the plant, i.e. on a part of a leaf, a root, a stem or another part of the plant.

Typically, with direct infection of plant tissues by *Agrobacterium* carrying the promoter and/or the GOI, a plant to be infected is wounded, e.g. by cutting the plant with a razor or puncturing the plant with a needle or rubbing the plant with an abrasive. The wound is then inoculated with the *Agrobacterium*. The inoculated plant or plant part is then grown on a suitable culture medium and allowed to develop into mature plants.

When plant cells are constructed, these cells may be grown and maintained in accordance with well-known tissue culturing methods such as by culturing the cells in a suitable culture medium supplied with the necessary growth factors such as amino acids, plant hormones, vitamins, etc.

Regeneration of the transformed cells into genetically modified plants may be accomplished using known methods for the regeneration of plants from cell or tissue cultures, for example by selecting transformed shoots using an antibiotic and by subculturing the shoots on a medium containing the appropriate nutrients, plant hormones, etc.

Further teachings on plant transformation may be found in EP-A-0449375.

In summation, the present invention provides an arabinofuranosidase enzyme having arabinoxylan degrading activity and the nucleotide sequence coding for the same. In addition, it provides a promoter that can control the expression of that, or another, nucleotide sequence. In addition it includes terminator and signal sequences for the same.

The following sample was deposited in accordance with the Budapest Treaty at the recognised depositary The National Collections of Industrial and Marine Bacteria Limited (NCIMB) at 23 St. Machar Drive, Aberdeen, Scotland, United Kingdom, AB2 1RY on 16 January 1995:

> *E.coli* containing plasmid pB53.1 {i.e. E.coli DH5α-pB53.1}. The deposit number is NCIMB 40703.

The present invention will now be described by way of example.

In the following Examples reference is made to the accompanying figures in which:

Figures 1-10 are sequences of promoters and GOIs of earlier patent applications that are useful for use with the aspects of the present invention;

Figure 11 is a plasmid map of the plasmid pB53.1, which is the subject of deposit NCIMB 40703;

Figure 12 is a schematic diagram of deletions made to the promoter of the present invention;

Figure 13 is a plasmid map of pXP-AMY;

Figure 14 is a plasmid map of pXP-XssAMY;

Figure 15 is a graph;

Figure 16 is an HP-TLC profile;

Figure 17 is an HP-TLC profile;

Figure 18 is an HPLC profile;

Figure 19 is a viscosity plot;

Figure 20 is an activity plot;

Figure 21 is an activity plot; and

Figure 22 is an activity plot.

The following text discusses the use of *inter alia* recombinant DNA techniques. General teachings of recombinant DNA techniques may be found in Sambrook, J., Fritsch, E.F., Maniatis T. (Editors) Molecular Cloning. A laboratory manual. Second edition. Cold

Spring Harbour Laboratory Press. New York 1989.

In these Examples, the enzyme of the present invention is sometimes referred to as AbfC. In addition, the promoter of the present invention is sometimes referred to as the AbfC promoter.

Purification of the arabinofuranosidase

Aspergillus niger 3M43 was grown in medium containing wheat bran and beet pulp. The fermentation broth was separated from the solid part of the broth by filtration. Concentrated fermentation broth was loaded on a 25X100mm Q-SEPHAROSE (Pharmacia) high Performance column, equilibrated with 20 mM Tris, HCl pH 7.5, and a linear gradient from 0-500 Mm NaCl was performed and fractions of the eluate was collected. The Arabinofuranosidase was eluted at 130-150 Mm NaCl.

The fractions containing the arabinofuranosidase were combined and desalted using a 50x200 mm G-25 SEPHAROSE Superfine (Pharmacia). The column was eluted with distilled water.

After desalting the enzyme was concentrated using High-Trap spin columns. Next the concentrated and desalted fractions were subjected to gel filtration on a 50x600 mm SUPERDEX 50 column. The sample was loaded and the column was eluted with 0.2 M Phosphate buffer pH 7.0 plus 0.2 M NaCl, and fractions of the eluate were collected.

The fractions containing arabinofuranosidase were combined and desalted and concentrated as described above. The combined fractions were loaded on a 16X100 mm Phenylsepharose High Performance column (Pharmacia), equilibrated with 50 mM Phosphate buffer pH 6.0, containing 1.5 M $(NH_4)_2SO_4$. A gradient where the $(NH_4)_2SO_4$ concentration was varied from 1.5 - 0 M was applied and the eluate collected in fractions. The fractions containing Arabinofuranosidase were combined. The purity of the arabinofuranosidase was evaluated by SDS-PAGE using the Phast system gel (Pharmacia).

Characterization

The molecular weight of the purified arabinofuranosidase was determined by mass spectrometry using laser desorption technology. The MW of the arabinofuranosidase was found to be 33.270 D ± 50 D.

The pI value was determined by use of a Broad pI Kit (Pharmacia). The arabinofuranosidase has a pI value of about 3.7.

After SDS-PAGE analysis, treatment PAS reagent showed that the arabinofuranosidase was glycosylated. The PAS staining was done according to the procedure of I. Van-Seuningen and M. Davril (1992) Electrophoresis **13** pp 97-99.

Activity Studies

Activity of AbfC as a function of water soluble pentosan (WSP) concentrations (mg/ml) was determined. The results are shown in Figure 21. The results show that AbfC activity reached maximum at substrate concentration of 8 mg/ml WSP.

pH Activity Studies

The effect of pH on the activity of the arabinofuranosidase of the present invention was investigated using water soluble pentosan (10 mg/ml) from wheat as a substrate in 50 mM citric acid sodium phosphate buffer. The incubation time was 15 minutes. The arabinofuranosidase of the present invention was observed to have a wide pH optima range of from about 2.5 to about 7.0 (see Figure 20), more especially from about 3.3 to about 4.6, more especially about 4.

Temperature Activity Studies

The effect of temperature on the activity of the arabinofuranosidase of the present invention was investigated using water soluble pentosan (10 mg/ml) from wheat as a substrate in 50 mM sodium acetate at a pH of 5.0. The incubation time was 15 minutes.

The arabinofuranosidase of the present invention was observed to have an optimal activity at a temperature of from about 40°C to about 60°C, more especially from about 45°C to about 55°C, more especially about 50°C (Figure 22). The enzyme is still active at about 10°C and showed residual activity at 70°C and 80°C.

Amino acid sequencing of the arabinofuranosidase

The enzyme was digested with endoproteinase Lys-C sequencing grade from Boehringer Mannheim using a modification of the method described by Stone & Williams 1993 (Stone, K.L. and Williams, K.R. (1993). Enzymatic digestion of Proteins and HPLC Peptide Isolation. In : Matsudaira P. (Editor). A practical Guide to Protein and Peptide Purification for Microsequencing. Second Edition. Academic Press, San Diego 1993. pp 45-73).

Freeze dried β-arabinofuranosidase (0.4 mg) was dissolved in 50 µl of 8M urea, 0.4 M NH_4HCO_3, pH 8.4. After overlay with N_2 and addition of 5 µl of 45 Mm DTT, the protein was denatured and reduced for 15 min at 50°C under N_2. After cooling to RT, 5 µl of 100 Mm iodoacetamide was added for the cysteines to be derivatised for 15 min at RT in the dark under N_2. Subsequently, 90 µl of water and 5 µg of endoproteinase Lys-C in 50 µl of 50 Mm Tricine and 10 mM EDTA, pH 8.0, was added and the digestion was carried out for 24h at 37°C under N_2. The resulting peptides were separated by reversed phase HPLC on a VYDAC C18 column (0.46 x 15 cm; 10 µm; The Separations Group; California) using solvent A: 0.1% TFA in water and solvent B: 0.1% TFA in acetonitrile. Selected peptides were rechromatographed on a Develosil C18 column (0.46 x 10 cm; 3µm) using the same solvent system prior to sequencing on an Applied Biosystems 476A sequencer using pulsed-liquid fast cycles.

The following peptide sequences were found:

SEQ I.D. No. 4

SEQ I.D. No. 5

SEQ I.D. No. 6

SEQ I.D. No. 7

SEQ I.D. No. 8

Isolation of a PCR clone of a fragment of the gene

PCR primers were synthesised using an Applied Biosystems DNA synthesiser model 392. In this regard, PCR primers were synthesized from one of the found peptide sequences, namely SEQ ID No. 5. The primers were:

One primer from EMTAQA (reversed)

SEQ ID NO. 9 GCY TGN GCN GTC ATY TC

17 mer 64 mix

One primer from MIVEAIG

SEQ ID NO. 10 ATG ATH GTN GAR GCN ATH GG

20 mer 288 mix

PCR amplification was performed with 100 pmol of each of these primers in 100 μl reactions using Amplitaq polymerase (PERKIN ELMER). The following program was:

STEP	TEMP	TIME
1	94°C	2 min
2	94°C	1 min
3	55°C	2 min
4	72°C	2 min
5	72°C	5 min
6	5°C	SOAK

Steps 2-4 were repeated for 40 cycles.

PCR reactions were performed on a PERKIN ELMER DNA Thermal Cycler.

A 100 bp amplified fragment was isolated and cloned into a pT7-Blue T-vector, according to the manufacturers instructions (Novagen).

Isolation of *A. niger* genomic DNA

1g. of frozen *A. niger* mycelium was ground in a mortar under liquid nitrogen. Following evaporation of the nitrogen cover, the ground mycelium was extracted with 15ml of an extraction buffer (100mM Tris·Hcl, pH 8.0, O.50mM EDTA, 500mM NaCl, 10mM ß-mercaptoethanol) containing 1ml 20% sodium dodecyl sulphate. After incubation at 65°C for 10 min. 5ml 5M KAc. pH 5.0, was added and the mixture further incubated, after mixing, on ice for 20 mins. After extraction, the mixture was centrifuged for 20 mins. and the supernatant mixed with 0.6 vol. isopropanol to precipitate the extracted DNA. After further centrifugation for 15 mins. the DNA pellet was dissolved in 0.7 ml TE (10mM Tris, HCl pH 8.0, 1mM EDTA) and precipitated with 75 µl 3M NaAc, pH 4.8, and 500 µl isopropanol.

After centrifugation the pellet was washed with 70% ETOH and dried under vacuum. The DNA was dissolved in 200 µl TE and stored at -20°C.

Construction of a library

20 µg genomic DNA was partly digested with Tsp509I, which gives ends which are compatible with *EcoR*I ends. The digested DNA was separated on a 1 % agarose gel and fragments of 4-10 kb was purified. A λZAPII *EcoR*I/CIAP kit from Stratagene was used for library construction according to the manufacturers instructions. 2 µl of the ligation (totally 5 µl) was packed with Gigapack Gold II packing extract from Stratagene. The library contained 650,000 independent clones.

Screening of the library

2 X 50.000 pfu was plated on NZY plates and plaquelifts were done on Hybond N sheets (Amersham). Plaquelifts were done in duplicates. The sheets were hybridized with the PCR clone labelled with ^{32}P dCTP (Amersham) using Ready-to-go labelling kit from Pharmacia. Positive clones were reckoned only when hybridization was detected on both sheets. The gene was sequenced, and the found sequence showed that all of the peptides sequenced were coded by the found sequence.

Sequence information

SEQ. ID. No. 12 presents the promoter sequence, the enzyme coding sequence, the terminator sequence and the signal sequence and the amino acid sequence of the enzyme of the present invention.

Arabinofuranosidase assay

Two different arabinoxylan preparations from wheat flour, Wheat Insoluble Pentosan (WIP) and Wheat soluble Pentosan (WSP), were degraded with the arabinofuranosidase enzyme of the present invention alone and in combination with an endoxylanase purified from *A. niger*. The assays were done on 1% substrate in 50 Mm 50 Mm Na-acetate buffer at pH 5.0. The reactions were performed at 30 °C for 2.5 hours. The reactions were stopped by addition of 3 vol. ethanol which precipitates the high molecular weight material. The samples were centrifuged and the supernatants were collected, dried under vacuum and resuspended in 0.5 ml distilled water. The samples were diluted 1:1 in water and analysed on a Chromopack Carbohydrate Pb column (300X7.8 mm, cat. 29010) using Shimadzu C-R4A Chromatopac HPLC system using a Shimadzu RI D-6A refractive index detector in accordance with the suppliers instructions.

The column was calibrated using a standard composed of 0.48 mg/ml xylotriose, 0.48 mg/ml xylobiose, 0.60 mg/ml xylose and 0.58 mg/ml L-arabinose. The peaks were identified and quantified using the software supplied with the equipment.

Results - Liberated saccharides from Wheat Insoluble Pentosan

Substrate 1% WIP in 50 Mm Na-acetate buffer pH 5.0. Values are expressed in mg/ml.

	xylotriose	xylobiose	xylose	arabinose
no enzyme	0.0	0.0	0.0	0.0
abfC	0.0	0.0	0.0	0.11
xyl	0.09	0.14	0.0	0.0
abfC + xyl	0.37	0.41	0.0	0.30

abfC denotes the enzyme according to the present invention; and xyl denotes the xylanase described before.

Results - Saccharides liberated from Wheat Soluble Pentosan

Substrate 1 % WSP in 50 Mm Na-acetate buffer pH 5.0. Values are expressed in mg/ml.

	xylotriose	xylobiose	xylose	arabinose
no enzyme	0.0	0.0	0.0	0.0
abfC	0.0	0.0	0.0	0.30
xyl	0.08	0.14	0.0	0.0
abfC + xyl	0.42	0.47	0.0	0.42

abfC denotes the enzyme according to the present invention; and xyl denotes the xylanase described before.

Figure 17 shows HP-TLC profiles of the AbfC enzyme acting synergistically with Xylanase A. In this Figure, the following abbreviations are used: water-soluble pentosan (WSP); water-insoluble pentosan (WIP); and oat xylan as substrate. The standards were: X- xylose; X_2- xylobiose; X_3- xylotriose; A- arabinose.

Figure 18 shows the HPLC analysis of hydrolysis products using 1% oat spelt xylan as the substrate. Figure 18(a) and Figure 18(b) show the products when the AbfC enzyme and the xylanase enzyme respectively were used alone. Figure 18(c) show the products when the AbfC enzyme and the xylanase enzyme when combined.

The results of these experiments provide two important findings.

First the enzyme of the present invention liberates arabinose, in particular L-arabinose, from arabinoxylan.

Second the combined actions of the enzyme according to the present invention with the endoxylanase is significantly higher than the sum of their individual action. Accordingly, the two enzymes affect each others enzymatic activities in a synergistic fashion.

Induction of the AbfC gene: Identification of inducers

The regulation of transcription of the AbfC encoding gene of *Aspergillus niger* was studied using a strain containing a fusion of the AbfC promoter to the β-glucuronidase encoding gene (uid A) of *E coli*.

GUS producing transformants were grown on different carbon sources and assayed both qualitatively and quantitatively for the ability to hydrolyse p-nitrophenol glucuronide.

The results are shown below:

CARBON SOURCE (1%)	GUS ACTIVITY AFTER 24 HOURS INDUCTION (units/mg)
xylose	12.37
xylitol	1.49
arabinose	6.66
arabitol	5.30
glucose	0.70
cellubiose	0.95
xylo-oligomer 70	17.26
glucopyranoside	0.40
methyl-xylopyranoside	24.20
xyloglucan	1.00
pectin	0.27
arabinogalactan	2.60
arabitol + glucose	2.20

The results show that the AbfC promoter is switched on after 24 hours when grown in the presence of xylose, xylo-oligomer 70, methyl-xylopyranoside, arabinose and arabitol. These studies also suggest that methyl-xylopyranoside is the natural and strongest inducer of this promoter.

The AbfC promoter is strongly repressed by glucose and is therefore under carbon catabolite repression. However, unlike all the published promoters for arabinofuranosidases, which are induced by arabinose and arabitol, the AbfC promoter of the present invention is regulated strongly by the intermediates in xylose metabolism. Accordingly, the present invention also covers an arabinofuranosidase promoter wherein the promoter is inducible by an intermediate in xylose metabolism.

Effects of different promoter deletions on the regulation of the expression of the AbfC gene

To study the regulation at the molecular level, experiments were set up to detect possible upstream regulating sequences required for expression of the AbfC gene. A series of plasmids with deletions in the 5' upstream region of the gene was constructed (see Figure 12). The *E coli* uid A gene was used as the reporter gene and a qualitative GUS assay was performed.

The results indicated that the truncated AbfC promoter of 590 bp contains sufficient information for the inducibility of the AbfC gene and its regulation. Deletion of 100 bps sequence from the *Xma* 111 to the *BamH*1 sites of the promoter led to a reduction in activity of this promoter. Therefore, this 100 bps area is important for good levels of gene expression. Deletion of 290 bps before the ATG identified this region to be important but not sufficient to abolish the activity of this promoter. All the transformants analysed containing this promoter construct showed very pale blue when tested (+- GUS). This region is as follows:

-170 T C A T C C A A T A T

As seen, this region contains the CCAAT element and is a putative target for a general transcriptional activator. This sequence is similar to the nuclear protein binding sites found in two starch inducible promoters: the *Aspergillus niger* glucoamylase gene and the *Aspergillus oryzae* amylase gene as well as the amdS gene of *Aspergillus nidulans*.

HETEROLOGOUS PROTEIN PRODUCTION USING *ASPERGILLUS NIGER* TRANSFORMED WITH THE AbfC PROMOTER AND THE AbfC SIGNAL SEQUENCE

Transformation of *Aspergillus Niger*

The protocol for transformation of *A. niger* was based on the teachings of Buxton,F.P., Gwynne D.I., Davis,R.W. 1985 (Transformation of *Aspergillus niger* using the *argB* gene of *Aspergillus nidulans*. Gene 37:207-214), Daboussi,M.J., Djeballi,A., Gerlinger, C., Blaiseau, P.L., Cassan, M., Lebrun, M.H., Parisot, D., Brygoo,Y. 1989 (Transformation of seven species of filamentous fungi using the nitrate reductase gene of *Aspergillus nidulans*. Curr. Genet. 15:453-456) and Punt, P.J., van den Hondel, C.A.M.J.J. 1992 (Transformation of filamentous fungi based on hygromycin B and Phleomycin resistance markers. Meth. Enzym. 216:447-457).

For the purification of protoplasts, spores from one PDA (Potato Dextrose Agar - from Difco Lab. Detroit) plate of fresh sporulated N400 (CBS 120.49, Centraalbureau voor Schimmelcultures, Baarn) (7 days old) are washed off in 5-10 ml water. A shake flask with 200 ml PDC (Potato Dextrose Broth, Difco 0549-17-9, Difco Lab. Detroit) is inoculated with this spore suspension and shaken (250 rpm) for 16-20 hours at 30°C.

The mycelium is harvested using Miracloth paper and 3-4 g wet mycelium are transferred to a sterile petri dish with 10 ml STC (1.2 M sorbitol, 10 mM Tris Hcl pH 7,5, 50 Mm $CaCl_2$) with 75 mg lysing enzymes (Sigma L-2265) and 4500 units lyticase (Sigma L-8012).

The mycelium is incubated with the enzyme until the mycelium is degraded and the protoplasts are released. The degraded mycelium is then filtered through a sterile 60 μm mesh filter. The protoplasts are harvested by centrifugation 10 min at 2000 rpm in a swing out rotor. The supernatant is discarded and the pellet is dissolved in 8 ml 1.5 M $MgSO_4$, and then centrifuged at 3000 rpm for 10 min.

The upper band, containing the protoplasts is transferred to another tube, using a transfer pipette and 2 ml 0.6 M KCl is added. Carefully 5 ml 30% sucrose is added on the top and the tube is centrifuged 15 min at 3000 rpm.

The protoplasts, lying in the interface band, are transferred to a new tube and diluted with 1 vol. STC. The solution is centrifuged 10 min at 3000 rpm. The pellet is washed twice with STC, and finally solubilized in 1 ml STC. The protoplasts are counted and eventually concentrated before transformation.

For the transformation, 100 μl protoplast solution (10^6-10^7 protoplasts) are mixed with 10 μl DNA solution containing 5- 10 μg DNA and incubated 25 min at room temperature. Then 60 % PEG-4000 is carefully added in portions of 200 μl, 200 μl and 800 μl. The mixture is incubated 20 min at room temperature. 3 ml STC is added to the mixture and carefully mixed. The mixture is centrifuged 3000 rpm for 10 min.

The supernatant is removed and the protoplasts are solubilized in the remaining of the supernatant. 3-5 ml topagarose is added and the protoplasts are quickly spread on selective plates.

AbfC promoter and heterologous gene expression

The expression vector pXP-Amy (Figure 13) contains the 2.1 kb α-amylase encoding gene from *Thermomyces lanuginosus* cloned downstream of the AbfC promoter (2.1 kb) and upstream of the Xylanase A terminator. This vector together with the hygromycin gene as a selectable marker was used for co-transformation experiments to test the functionality of the AbfC promoter.

The best transformant was accumulated in shake flask experiments at least 1 gram per litre of α-amylase in the culture media. Starch degrading activity was then detected within 48 hours and a peak of enzyme activity is observed at 4 days of growth on sugar beet pulp and wheat bran (Figure 15).

AbfC signal sequence functions in protein secretion

An expression construct containing the signal peptide of the AbfC gene translationally fused to the mature α-amylase from *T. lanuginosus* was prepared and expression of this construct in the production strains was observed. In this regard, the translational fusion construct pXPXss-Amy (Figure 14) was placed under the transcriptional control of the AbfC promoter and the xylanase A termination signal. The incorporation of an endogenous signal peptide resulted in increased detectability of co-transformants expressing both amylase and the hygromycin resistance marker. The endogenous signal peptide directed the secretion of amylase out of the cell.

Substrate Specificity of AbfC Protein

The substrate specificity of the purified AbfC was determined using arabinose containing hemicelluloses: arabinoxylans from wheat, oat and larch, branched and debranched arabinans; arabinogalactan, sugar beet pectin, and xyloglucan.

The HPLC and HP-TLC results are shown in Figure 16, in which the following abbreviations are used: WSP - water-soluble pentosan, WIP - water-insoluble pentosan, AG - arabinogalactan, deB-A - debranched arabinan. The standards used were: A-arabinose, X- xylose.

The results indicate that arabinose is the hydrolysis product from arabinoxylans. No hydrolysis products were released from arabinogalactan, debranched arabinan or xyloglucan. Arabinose was released as a hydrolysis product from branched arabinan. AbfC is therefore a 1,2/1,2 debranching enzyme and it has no activity towards linear 1,5 α-linked L-arabinofuranose residues found in debranched arabinans and arabinogalactan. This enzyme also releases a product when pectin is used as the substrate. It is believed that this product is an arabinose containing ferulic acid or an arabinobiose.

Reduction of Viscosity By AbfC

The results for the substrate specificity studies also suggest that the enzyme of the present invention could be used to reduce the viscosity of feeds. In this regard, the enzyme would reduce the viscosity of branched substrates by removing the branches but not the backbone of that substrate. This is in contrast to the known viscosity modifiers which degrade the substrate backbone.

Accordingly, the present invention covers a process of reducing the viscosity of a branched substrate wherein the enzyme degrades the branches of the substrate but not the backbone of the substrate.

In particular, the present invention covers the use of the enzyme of the present invention as a viscosity modifier.

In this regard, an experiment was carried out to investigate the reduction of viscosity of the water-soluble pentosan fraction from wheat flour by arabinofuranosidase. In this experiment, 6 ml water-soluble pentosan was incubated with 100 μl of AbfC for 20 hours, 20°C at pH 5.5.

The results (see Figure 19) show that the enzyme of the present invention can be used to reduce the viscosity of pectins, especially pectins that are used in beverages - such as fruit juices.

Accordingly, the present invention covers the use of the enzyme of the present invention to reduce the viscosity of pectin.

ANTIBODY PRODUCTION

Antibodies were raised against the enzyme of the present invention by injecting rabbits with the purified enzyme and isolating the immunoglobulins from antiserum according to procedures described according to N Harboe and A Ingild ("Immunization, Isolation

of Immunoglobulins, Estimation of Antibody Titre" In A Manual of Quantitative Immunoelectrophoresis, Methods and Applications, N H Axelsen, *et al* (eds.), Universitetsforlaget, Oslo, 1973) and by T G Cooper ("The Tools of Biochemistry", John Wiley & Sons, New York, 1977).

SUMMARY

Even though it is known that *Aspergillus niger* produces arabinofuranosidases, the present invention provides a novel and inventive arabinofuranosidase, as well as the coding sequence therefor and the promoter for that sequence. An important advantage of the present invention is that the enzyme can be produced in high amounts.

In addition, the promoter and the regulatory sequences (such as the signal sequence and the terminator) can be used to express or can be used in the expression of GOIs in organisms, such as in *A. niger*.

The arabinofuranosidase of the present invention is different from the arabinofuranosidases previously known. In this regard, the previous described arabinofuranosidases - such as those of EP-A-0506190 - are characterised by their ability to degrade arabinan, and are assayed using p-nitrophenyl-arabinoside.

The arabinofuranosidase of the present invention does not degrade arabinan, and only a minor activity is seen on p-nitrophenyl-arabinoside.

In contrast, the arabinofuranosidase of the present invention is useful for degrading arabinoxylan. Therefore, the arabinofuranosidase of the present invention is quite different from the previous isolated arabinofuranosidases.

More in particular, the enzyme of the present invention is capable of specifically cleaving arabinose from the xylose backbone of arabinoxylan.

The enzyme of the present invention is useful as it can improve processes for preparing foodstuffs and feeds as well as the foodstuffs and feeds themselves. For example, the enzyme of the present invention may be added to animal feeds which are rich in arabinoxylans. When added to feeds (including silage) for monogastic animals (e.g. poultry or swine) which contain cereals such as barley, wheat, maize, rye or oats or cereal by-products such as wheat bran or maize bran, the enzyme significantly improves the break-down of plant cell walls which leads to better utilization of the plant nutrients by the animal. As a consequence, growth rate and/or feed conversion are improved. Moreover, arabinoxylan-degrading enzymes may be used to reduce the viscosity of feeds containing arabinans. The arabinoxylan-degrading enzyme may be added beforehand to the feed or silage if pre-soaking or wet diets are preferred.

Of particular benefit is the use of the enzyme according to the present invention in combination with a xylanase, especially an endoxylanase.

A possible further application for the enzyme according to the present invention is in the pulp and paper industry. The application of xylanases is often reported to be beneficial in the removal of lignins and terpenoids from the cellulose and hemicellulose residues of a hemicellulose backbone, an essential step in the processing of wood, wood pulp or wood derivative product for the production of paper. The addition of arabinoxylan-degrading enzymes, produced according to the present invention, to the xylanase treatment step should assist in the degradation of an arabinan-containing hemicellulose backbone and thus facilitate an improved, more efficient removal of both lignins and terpenoids. The application of arabinoxylan-degrading enzymes should be particularly advantageous in the processing of soft woods in which the hemicellulose backbone contains glucuronic acid.

The enzyme according to the present invention is also useful as it acts in a synergistic manner with endoxylanase (see results presented above).

Other modifications of the present invention will be apparent to those skilled in the art without departing from the scope of the invention.

SEQUENCE LISTINGS

SEQ ID NO: 1

ENZYME SEQUENCE

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 296 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Lys Cys Ser Leu Pro Ser
1               5
Ser Tyr Ser Trp Ser Ser Thr Asp Ala Leu Ala Thr Pro Lys Ser Gly
            10                  15                  20
Trp Thr Ala Leu Lys Asp Phe Thr Asp Val Val Ser Asp Gly Lys His
        25                  30                  35
Ile Val Tyr Ala Ser Thr Thr Asp Glu Ala Gly Asn Tyr Gly Ser Met
    40                  45                  50
Thr Phe Gly Ala Phe Ser Glu Trp Ser Asn Met Ala Ser Ala Ser Lys
55                  60                  65                  70
Thr Ala Thr Pro Tyr Asn Ala Val Ala Pro Thr Leu Phe Tyr Phe Lys
                75                  80                  85
Pro Lys Ser Ile Trp Val Leu Ala Tyr Gln Trp Gly Ser Ser Thr Phe
            90                  95                  100
Thr Tyr Arg Thr Ser Gln Asp Pro Thr Asn Val Asn Gly Trp Ser Ser
        105                 110                 115
Glu Lys Ala Leu Phe Thr Gly Lys Leu Ser Asp Ser Ser Thr Gly Ala
    120                 125                 130
Ile Asp Gln Thr Val Ile Gly Asp Asp Thr Asn Met Tyr Leu Phe Phe
135                 140                 145                 150
Ala Gly Asp Asn Gly Lys Ile Tyr Arg Ser Ser Met Ser Ile Asp Glu
                155                 160                 165
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Phe Pro Gly Ser Phe Gly Ser Gln Tyr Glu Glu Ile Leu Ser Gly Ala
            170                 175                 180
Thr Asn Asp Leu Phe Glu Ala Val Gln Val Tyr Thr Val Asp Gly Gly
        185                 190                 195
Glu Gly Asn Ser Lys Tyr Leu Met Ile Val Glu Ala Ile Gly Ser Thr
    200                 205                 210
Gly His Arg Tyr Phe Arg Ser Phe Thr Ala Ser Ser Leu Gly Gly Glu
215                 220                 225                 230
Trp Thr Ala Gln Ala Ala Ser Glu Asp Lys Pro Phe Ala Ala Lys Pro
                235                 240                 245
Thr Val Ala Pro Pro Gly Pro Lys Thr Leu Ala Met Val Thr Trp Phe
            250                 255                 260
Ala Thr Thr Leu Ile Lys Pro  *
        265                 270
```

SEQ ID NO: 2
NUCLEOTIDE CODING SEQUENCE

AAA TGC TCT CTT CCA TCG TCC TAT AGT TGG AGT TCA ACC GAT GCT CTC
GCA ACT CCT AAG TCA GGA TGG ACC GCA CTG AAG GAC TTT ACT GAT GTT
GTC TCT GAC GGC AAA CAT ATC GTC TAT GCG TCC ACT ACT GAT GAA GCG
GGA AAC TAT GGC TCG ATG ACC TTT GGC GCT TTC TCA GAG TGG TCG AAC
ATG GCA TCT GCT AGC AAG ACA GCC ACC CCC TAC AAT GCC GTG GCT CCT
ACC CTG TTC TAC TTC AAG CCG AAA AGC ATC TGG GTT CTG GCC TAC CAA
TGG GGC TCC AGC ACA TTC ACC TAC CGC ACC TCC CAA GAT CCC ACC AAT
GTC AAC GGC TGG TCG TCG GAG AAG GCG CTT TTC ACC GGA AAA CTC AGC
GAC TCA AGC ACC GGT GCC ATT GAC CAG ACG GTG ATT GGC GAC GAT ACG
AAT ATG TAT CTC TTC TTT GCT GGC GAC AAC GGC AAG ATC TAC CGA TCC
AGC ATG TCC ATC GAT GAA TTT CCC GGA AGC TTC GGC AGC CAG TAC GAG
GAA ATT CTG AGT GGT GCC ACC AAC GAC CTA TTC GAG GCG GTC CAA GTG
TAC ACG GTT GAC GGC GGC GAG GGC AAC AGC AAG TAC CTC ATG ATC GTT
GAG GCG ATC GGG TCC ACT GGA CAT CGT TAT TTC CGC TCC TTC ACG GCC
AGC AGT CTC GGT GGA GAG TGG ACA GCC CAG GCG GCA AGT GAG GAT AAA
CCC TTC GCA GCA AAG CCA ACA GTG GCG CCA CCT GGA CCG AAG ACA TTA
GCC ATG GTG ACT TGG TTC GCA ACA ACC CTG ATC AAA CCA TGA

SEQ ID NO: 3
PROMOTER SEQUENCE

CTGCAGAAGA TGGCAGTCGC CACAGCCGAT CACCCGATCC ATACTGGATG TTGTAACTTG 60

GAGACAGCCT GCAGATGCTC TGATGAAGGT CTGCAAATAG TTCCTGGACC TCGATAGTGA 120

AGTATACCGA TTCGTCAATG TTGTATATCC AGCCACTTTG AAAGTACCAA CTTTTAGTTC 180

GATTGATCAG AATACTTTTG GTGTGTAACA TTGACAAGCC AAATTATCAA TCTCTTCTAC 240

CGGTAAGGTG TCAACTACCC GGCCGAAAGT ACCGGAAGGT CGTGGTGTTT TAAGGTGAAA 300

CAACTATCAG GGCGGCAATG TGTCAAAGTA GAACCAGTTT GCTTAGCGCC ATTAGGATCC 360

ACGCCTAGAC CCTTGATGCC CGGGAGTTAT CCGTCCTGTC ACAGCAATTA TTTCCCCGAG 420

TCTACTGCCG AAGAACAGCC ATTGTGGCGT ACTCACGGAA TTACCCACTG TGTAGGGTAG 480

TCTTGAACGC CGTTCTAGAC ACGGCAACGC TCCGGTGGAC GATCGTTTCT GGCTAATGTA 540

CTCCGTAGTT TAGGCAGCAT GCTGATCATC TTCCCCCTAG GGAAAGGCCC CTGAATAGTG 600

CGCCAAAATG AGCTTGAGCA AAGGAATGTT CTTTCTAAGC CAAAGTGAGG GAAATAACCA 660

AGCAGCCCAC TTTTATCCGA AACGTTTCTG GTGTCATCCA ATATGGATAA ATCCCGATTG 720

TTCTTCTGCA CATATCTCTA TTGTCATAAG TGCAACTACA TATATTTGAA CATGGTTTGG 780

TCCTCTTTCC AAGTTATTCG TTCTCCGTGA CCAGCGATTT CAGCCATTGA TTCTTTTGTT 840

TCTTTCCCCG CGGATAAACT CATACGAAG

INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: N-terminal
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Lys Cys Ser Leu Pro Ser Ser Tyr Ser Trp Ser Ser Thr Asp Ala Leu
1 5 10 15
Ala Thr Pro Lys
20

INFORMATION FOR SEQ ID NO: 5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
Tyr Leu Met Ile Val Glu Ala Ile Gly Ser Thr Gly His Arg Tyr Phe
1 5 10 15
Arg Ser Phe Thr Ala Ser Ser Leu Gly Gly Glu Met Thr Ala Gln Ala
20 25 30
Ala Ser Glu Asp Lys Pro Phe Xaa Gly
35 40

INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Ser Ile Trp Val Leu Ala Tyr Gln Trp Gly Ser Ser Thr Phe Thr Tyr
1 5 10 15

Arg Thr Ser Gln Asp Pro Thr Asn Val
20 25

INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Asp Ile Val Tyr Ala Ser Thr Thr Asp Glu Ala Gly Asn Tyr Gly Ser
1 5 10 15

Met Thr Phe Gly Ala Phe Ser Glu Xaa Ser Asn Met Ala Ser
20 25 30

INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ile Tyr Arg Ser Ser Met Ser Ile Asp Glu Phe Pro Gly Ser Phe Gly
1 5 10 15

Ser Gln Tyr Glu Glu Ile Leu Ser Gly Ala Thr Asn Asp Leu Phe Glu
20 25 30

Ala Val Gln Val Tyr Thr Val Asp Gly
35 40

INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "OLIGONUCLEOTIDE"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GCYTGNGCNG TCATYTC 17

INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "OLIGONUCLEOTIDE"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

ATG ATH GTN GAR GCN ATH GG 20

INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 89 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "PCR fragment"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ATGATTGTGG AGGCGATCGG GTCCACTGGA CATCGTTATT TCCGCTCCTT CACGGCCAGC 60

AGTCTCGGTG GAGAGATGAC CGCACAGGC 89

INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2555 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Aspergillus niger

(B) STRAIN: 3M43

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:870..1757

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION:870..947

(ix) FEATURE:

(A) NAME/KEY: mat_peptide

(B) LOCATION:948..1754

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CTGCAGAAGA TGGCAGTCGC CACAGCCGAT CACCCGATCC ATACTGGATG TTGTAACTTG 60

GAGACAGCCT GCAGATGCTC TGATGAAGGT CTGCAAATAG TTCCTGGACC TCGATAGTGA 120

AGTATACCGA TTCGTCAATG TTGTATATCC AGCCACTTTG AAAGTACCAA CTTTTAGTTC 180

GATTGATCAG AATACTTTTG GTGTGTAACA TTGACAAGCC AAATTATCAA TCTCTTCTAC 240

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CGGTAAGGTG TCAACTACCC GGCCGAAAGT ACCGGAAGGT CGTGGTGTTT TAAGGTGAAA     300
CAACTATCAG GGCGGCAATG TGTCAAAGTA GAACCAGTTT GCTTAGCGCC ATTAGGATCC     360
ACGCCTAGAC CCTTGATGCC CGGGAGTTAT CCGTCCTGTC ACAGCAATTA TTTCCCCGAG     420
TCTACTGCCG AAGAACAGCC ATTGTGGCGT ACTCACGGAA TTACCCACTG TGTAGGGTAG     480
TCTTGAACGC CGTTCTAGAC ACGGCAACGC TCCGGTGGAC GATCGTTTCT GGCTAATGTA     540
CTCCGTAGTT TAGGCAGCAT GCTGATCATC TTCCCCCTAG GGAAAGGCCC CTGAATAGTG     600
CGCCAAAATG AGCTTGAGCA AAGGAATGTT CTTTCTAAGC CAAAGTGAGG GAAATAACCA     660
AGCAGCCCAC TTTTATCCGA AACGTTTCTG GTGTCATCCA ATATGGATAA ATCCCGATTG     720
TTCTTCTGCA CATATCTCTA TTGTCATAAG TGCAACTACA TATATTTGAA CATGGTTTGG     780
TCCTCTTTCC AAGTTATTCG TTCTCCGTGA CCAGCGATTT CAGCCATTGA TTCTTTTGTT     840
TCTTTCCCCG CGGATAAACT CATACGAAG ATG AAG TTC TTC AAT GCC AAA GGC        893
                                  Met Lys Phe Phe Asn Ala Lys Gly
                                  -26 -25                 -20
AGC TTG CTG TCA TCA GGA ATC TAC CTC ATT GCA TTA ACC CCC TTT GTT        941
Ser Leu Leu Ser Ser Gly Ile Tyr Leu Ile Ala Leu Thr Pro Phe Val
            -15                 -10                 -5
AAC GCC AAA TGC TCT CTT CCA TCG TCC TAT AGT TGG AGT TCA ACC GAT        989
Asn Ala Lys Cys Ser Leu Pro Ser Ser Tyr Ser Trp Ser Ser Thr Asp
         1               5                  10
GCT CTC GCA ACT CCT AAG TCA GGA TGG ACC GCA CTG AAG GAC TTT ACT       1037
Ala Leu Ala Thr Pro Lys Ser Gly Trp Thr Ala Leu Lys Asp Phe Thr
 15                  20                  25                  30
GAT GTT GTC TCT GAC GGC AAA CAT ATC GTC TAT GCG TCC ACT ACT GAT       1085
Asp Val Val Ser Asp Gly Lys His Ile Val Tyr Ala Ser Thr Thr Asp
                 35                  40                  45
GAA GCG GGA AAC TAT GGC TCG ATG ACC TTT GGC GCT TTC TCA GAG TGG       1133
Glu Ala Gly Asn Tyr Gly Ser Met Thr Phe Gly Ala Phe Ser Glu Trp
             50                  55                  60
TCG AAC ATG GCA TCT GCT AGC AAG ACA GCC ACC CCC TAC AAT GCC GTG       1181
Ser Asn Met Ala Ser Ala Ser Lys Thr Ala Thr Pro Tyr Asn Ala Val
         65                  70                  75
GCT CCT ACC CTG TTC TAC TTC AAG CCG AAA AGC ATC TGG GTT CTG GCC       1229
Ala Pro Thr Leu Phe Tyr Phe Lys Pro Lys Ser Ile Trp Val Leu Ala
     80                  85                  90
TAC CAA TGG GGC TCC AGC ACA TTC ACC TAC CGC ACC TCC CAA GAT CCC       1277
Tyr Gln Trp Gly Ser Ser Thr Phe Thr Tyr Arg Thr Ser Gln Asp Pro
 95                  100                 105                 110
```

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ACC AAT GTC AAC GGC TGG TCG TCG GAG AAG GCG CTT TTC ACC GGA AAA      1325
Thr Asn Val Asn Gly Trp Ser Ser Glu Lys Ala Leu Phe Thr Gly Lys
                115                 120                 125
CTC AGC GAC TCA AGC ACC GGT GCC ATT GAC CAG ACG GTG ATT GGC GAC      1373
Leu Ser Asp Ser Ser Thr Gly Ala Ile Asp Gln Thr Val Ile Gly Asp
            130                 135                 140
GAT ACG AAT ATG TAT CTC TTC TTT GCT GGC GAC AAC GGC AAG ATC TAC      1421
Asp Thr Asn Met Tyr Leu Phe Phe Ala Gly Asp Asn Gly Lys Ile Tyr
        145                 150                 155
CGA TCC AGC ATG TCC ATC GAT GAA TTT CCC GGA AGC TTC GGC AGC CAG      1469
Arg Ser Ser Met Ser Ile Asp Glu Phe Pro Gly Ser Phe Gly Ser Gln
    160                 165                 170
TAC GAG GAA ATT CTG AGT GGT GCC ACC AAC GAC CTA TTC GAG GCG GTC      1517
Tyr Glu Glu Ile Leu Ser Gly Ala Thr Asn Asp Leu Phe Glu Ala Val
175                 180                 185                 190
CAA GTG TAC ACG GTT GAC GGC GGC GAG GGC AAC AGC AAG TAC CTC ATG      1565
Gln Val Tyr Thr Val Asp Gly Gly Glu Gly Asn Ser Lys Tyr Leu Met
                195                 200                 205
ATC GTT GAG GCG ATC GGG TCC ACT GGA CAT CGT TAT TTC CGC TCC TTC      1613
Ile Val Glu Ala Ile Gly Ser Thr Gly His Arg Tyr Phe Arg Ser Phe
            210                 215                 220
ACG GCC AGC AGT CTC GGT GGA GAG TGG ACA GCC CAG GCG GCA AGT GAG      1661
Thr Ala Ser Ser Leu Gly Gly Glu Trp Thr Ala Gln Ala Ala Ser Glu
        225                 230                 235
GAT AAA CCC TTC GCA GCA AAG CCA ACA GTG GCG CCA CCT GGA CCG AAG      1709
Asp Lys Pro Phe Ala Ala Lys Pro Thr Val Ala Pro Pro Gly Pro Lys
    240                 245                 250
ACA TTA GCC ATG GTG ACT TGG TTC GCA ACA ACC CTG ATC AAA CCA TGA      1757
Thr Leu Ala Met Val Thr Trp Phe Ala Thr Thr Leu Ile Lys Pro  *
255                 260                 265                 270
CTGTCGATCC TTGCAACCTC CAGTTGCTCT ATCAGGGCCA TGACCCCCAA CAGCAGTGGC    1817
GACTACAACC TCTTGCCATG GAAGCCGGGC GTCCTTACCT TGAAGCAGTG ACGAGCTTAT    1877
CTTTAGTTGC AGATCGTGTT TCTCCTTTCT TCTTCAAGTA GTTTTAGTGG TGGAAGACAG    1937
CAGAAGGTGG TCATCATCTT AGGCTCAGTT GGGGTGGGCT CCTGCCACGT TTTGTCCATA    1997
GGCTAGTAAT TTGCACGGAA TTCAGTTCAT TGGCAAGGAG TGCGGTACGA ATACCTGTTT    2057
TCACAATAGC AATTAGGCCC AGTAGTTATA CTACGTACTG GAATTGAGTA CTCGTAGTAG    2117
CAAGATTGTT TGCCTCAGAG GGAATGGCCG ACACGTGAGC AAGTCACCTT CATCAGCTAG    2177
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TCGCGTTCCA CATAGACAAT GGTCCAGCTC CAGAGTGGAA TTTGGGCTAC TTTGAACGAT 2237
GGCCGATTGA ATCGCGCGTC TCCTCAATTG TATTTAACCA CAATAGGCCA GGTATTGGCA 2297
TTCACTCTCC GCCTTTGCGG GTGCCGGCAC GAGATGTCTC CTGAAGAAAC TAGGCAACGA 2357
GCAGACTGTG GATATGGGAG ATGGTTGACG ATGTGCTTCT TGGTAAATTT GAAGCCTCCA 2417
GGGCCTCTAG AAAGGCGGGA ATTTAAATCT CAAGTGCCCT AACGTGTCCG ACCACGGTGT 2477
TGATCATCAT TCATTGAATC GGATAACAGT CTTGGTTCGG AAACTGAACA GGCGGCTCTT 2537
GAATGACACT CTGGATCC 2555

(2) INFORMATION FOR SEQ ID NO: 13:
TERMINATOR SEQUENCE
CTGTCGATCC TTGCAACCTC CAGTTGCTCT ATCAGGGCCA TGACCCCCAA CAGCAGTGGC 60
GACTACAACC TCTTGCCATG GAAGCCGGGC GTCCTTACCT TGAAGCAGTG ACGAGCTTAT 120
CTTTAGTTGC AGATCGTGTT TCTCCTTTCT TCTTCAAGTA GTTTTAGTGG TGGAAGACAG 180
CAGAAGGTGG TCATCATCTT AGGCTCAGTT GGGGTGGGCT CCTGCCACGT TTTGTCCATA 240
GGCTAGTAAT TTGCACGGAA TTCAGTTCAT TGGCAAGGAG TGCGGTACGA ATACCTGTTT 300
TCACAATAGC AATTAGGCCC AGTAGTTATA CTACGTACTG GAATTGAGTA CTCGTAGTAG 360
CAAGATTGTT TGCCTCAGAG GGAATGGCCG ACACGTGAGC AAGTCACCTT CATCAGCTAG 420
TCGCGTTCCA CATAGACAAT GGTCCAGCTC CAGAGTGGAA TTTGGGCTAC TTTGAACGAT 480
GGCCGATTGA ATCGCGCGTC TCCTCAATTG TATTTAACCA CAATAGGCCA GGTATTGGCA 540
TTCACTCTCC GCCTTTGCGG GTGCCGGCAC GAGATGTCTC CTGAAGAAAC TAGGCAACGA 600
GCAGACTGTG GATATGGGAG ATGGTTGACG ATGTGCTTCT TGGTAAATTT GAAGCCTCCA 660
GGGCCTCTAG AAAGGCGGGA ATTTAAATCT CAAGTGCCCT AACGTGTCCG ACCACGGTGT 720
TGATCATCAT TCATTGAATC GGATAACAGT CTTGGTTCGG AAACTGAACA GGCGGCTCTT 780
GAATGACACT CTGGATCC 798

(2) INFORMATION FOR SEQ ID NO: 14
Signal SEQUENCE
ATG AAG TTC TTC AAT GCC AAA GGC AGC TTG CTG TCA TCA GGA ATC TAC 48
CTC ATT GCA TTA ACC CCC TTT GTT AAC GCC 78

SEQ ID NO: 15

SIGNAL SEQUENCE

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Met Lys Phe Phe Asn Ala Lys Gly Ser Leu 10

Leu Ser Ser Gly Ile Tyr Leu Ile Ala Leu 20

Thr Pro Phe Val Asn Ala 26

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 27, line s 25 and 26

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet: ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St. Machar Drive
Aberdeen
Scotland
AB2 1RY
United Kingdom

Date of deposit	Accession Number
16 JANUARY 1995	NCIMB 40703

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet: ☐

In respect of those designations in which a European patent is sought, and any other designated state having equivalent legislation, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer: J. van Aubel	Authorized officer:

Form PCT/RO/134 (July 1992)

CLAIMS

1. An enzyme that is obtainable from *Aspergillus*, wherein the enzyme has the following characteristics:

a. a MW of 33,270 D ± 50 D
b. a pI value of about 3.7
c. arabinoxylan degrading activity
d. a pH optima of from about 2.5 to about 7.0 (more especially from about 3.3 to about 4.6, more especially about 4)
e. a temperature optima of from about 40°C to about 60°C (more especially from about 45°C to about 55°C, more especially about 50°C);

wherein the enzyme is capable of cleaving arabinose from the xylose backbone of an arabinoxylan.

2. An enzyme having the sequence shown as SEQ. I.D. No. 1 or a variant, homologue or fragment thereof.

3. An enzyme coded by the nucleotide sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.

4. A nucleotide sequence coding for the enzyme according to claim 1.

5. A nucleotide sequence coding for the enzyme according to claim 2.

6. A nucleotide sequence having the sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.

7. A nucleotide sequence according to any one of claims 4 to 6 operatively linked to a promoter.

8. A nucleotide sequence according to claim 7 wherein the promoter is the promoter having the sequence shown as SEQ. I.D. No. 3 or a variant, homologue or fragment thereof or a sequence complementary thereto.

9. A promoter having the sequence shown as SEQ. I.D. No. 3 or a variant, homologue or fragment thereof or a sequence complementary thereto.

10. A promoter according to claim 9 operatively linked to a GOI.

11. A promoter according to claim 10 wherein the promoter is operatively linked to a GOI, wherein the GOI comprises a nucleotide sequence according to any one of claims 4-6.

12. A terminator having the nucleotide sequence shown as SEQ. I.D. No. 13 or a variant, homologue or fragment thereof or a sequence complementary thereto.

13. A signal sequence having the nucleotide sequence shown as SEQ. I.D. No. 14 or a variant, homologue or fragment thereof or a sequence complementary thereto.

14. A construct comprising or expressing the invention according to any one of claims 1 to 13.

15. A vector comprising or expressing the invention of any one of claims 1 to 14.

16. A plasmid comprising or expressing the invention of any one of claims 1 to 15.

17. A transgenic organism comprising or expressing the invention according to any one of claims 1 to 16.

18. A transgenic organism according to claim 17 wherein the organism is a fungus.

19. A transgenic organism according to claim 18 wherein the organism is a filamentous fungus, preferably of the genus *Aspergillus*.

20. A transgenic organism according to claim 17 wherein the organism is a plant.

21. A process of preparing an enzyme according to any one of claims 1 to 3 comprising expressing a nucleotide sequence according to any one of claims 4-8.

22. A process according to claim 21 wherein the enzyme has the sequence shown as SEQ. I.D. No. 1 or a variant, homologue or fragment thereof, and the nucleotide sequence has the sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.

23. A process according to claim 21 or claim 22 wherein the expression is controlled (partially or completely) by use of a promoter according to claim 9.

24. A process for expressing a GOI by use of a promoter, wherein the promoter is the promoter according to claim 9.

25. Use of an enzyme according to any one of claims 1 to 3 or prepared by a process according to any one of claims 21 to 24 to degrade an arabinoxylan.

26. Use according to claim 24 wherein the enzyme is used in combination with a xylanase, preferably an endoxylanase.

27. A combination of enzymes to degrade an arabinoxylan, the combination comprising an enzyme according to any one of claims 1 to 3 or prepared by a process according to any one of claims 21 to 24 claims; and a xylanase.

28. Plasmid NCIMB 40703, or a nucleotide sequence obtainable therefrom for expressing an enzyme capable of degrading arabinoxylan or for controlling the expression thereof or for controlling the expression of another GOI.

29. A signal sequence having the sequence shown as SEQ. I.D. No. 15 or a variant, homologue or fragment thereof.

30. The use of the enzyme according to any one of claims 1 to 3 or prepared by a process according to any one of claims 21 to 24 claims, in the manufacture of a medicament or foodstuff to reduce or prevent indigestion and/or increase nutrient absorption.

31. An arabinofuranosidase enzyme having arabinoxylan degrading activity, which is immunologically reactive with an antibody raised against a purified arabinofuranosidase enzyme having the sequence shown as SEQ. I.D. No. 1.

FIGURE 1

AMY 637 PROMOTER
SEQUENCE TYPE: Nucleotide
MOLECULE TYPE: DNA
ORIGINAL SOURCE: *Solanum Tuberosum*
SEQUENCE LENGTH: 2094
SEQUENCE:

```
        10         20         30         40
ATTAAGGGGA GCATAAGTGC AGCTCAGAAA TTCACACCTG
        50         60         70         80
ATATTTTCCC AAAGCCCTCA AAAATGTGAA CAAATCTGCT
        90        100        110        120
AAAATGTCAG TCAGAAGGAC TGTTCTTTTA GGTTTTCTTC
       130        140        150        160
TCTCGAGTCA CGAAATCAGA TAATATGATA AGAAATTATG
       170        180        190        200
GAGGATTTAT AATGTATCTG TCTGTTCTTA GGTATAATTA
       210        220        230        240
TGTGTTCCTT TATGATGTAG TAATGGAATT CTGGGCTTAT
       250        260        270        280
ATTAAAGGAA CTGAATATAA ATGTTCGCAT TTTAACTGCG
       290        300        310        320
GAGACTTCGA GTTAGAGCCT TATAATTATG TCTTATCATT
       330        340        350        360
TTATACTGAG ATCATATTAC AGATGATGAA AGCTGACATT
       370        380        390        400
GCATTAGTTA TTCTGTTTTA TACAAGTCAT GTAACTGCTG
       410        420        430        440
CTTGTGAGTT GTGACTGTAA GATAAATTGA TTCAGCCTTC
       450        460        470        480
TGTGGCATTA GCGGAGATCT GATTATACTC TCATCGTCTT
       490        500        510        520
ATCTAAGTTG CTCATGCAAC TTTGTCCTTG ATAGTTGGCT
       530        540        550        560
AATACTACAA CTGGAATTAA GTGTAGTTAT TCGAAATCTC
       570        580        590        600
TGTTGGAAGT TGCTAAGTGC TTAAGTGCTG GTTATTGTAA
       610        620        630        640
ACCCCATCCG AGTTATTATA CAGCATCTGG CTGATGAAAT
       650        660        670        680
GCTGCTCATT TGCAATGGTG ACATAACCAA ATGTTAGTAA
       690        700        710        720
AACATACTAG CTGGTTGAAT GTTAGATGAT TGTTCAACGT
       730        740        750        760
TACATCTCAC AGAAACCTTA TTATGGATTG ACATGTTAGT
```

FIGURE 1 CONTINUED

```
       770         780         790         800
TGATCCGAAA GATCCTTCTT TTAAATGCCA AAGCTTGTTA
       810         820         830         840
CAGATTTGAG GAGTTCTTTT ACTTTCTTTT GTTATATCTA
       850         860         870         880
TTTCCCATTC ATTTTGACGT TCAGCCTCAC AGATGTTGTC
       890         900         910         920
ATACTTAGAA ATGTGCGTAT ATATATAGAG AGAGAGAGAT
       930         940         950         960
AGAGTGAAAT GATTATATAG TCGAAGATTA CGAAACTTGA
       970         980         990        1000
CATTGAGACA TCTGTGATTG TTTGAAATTT ATGTATATAT
      1010        1020        1030        1040
CTGTAGCATT AGAAACTATA AGAGTTGTTA GCTTCACTTG
      1050        1060        1070        1080
TCTTATTGTT GTGCTCAAAG CAACTTCATC ATACAGTATG
      1090        1100        1110        1120
GTTTTTATAT GCTCTTCCAT TATCACCGAA CCTTATGATT
      1130        1140        1150        1160
ATGTGTACGA GCTTATAATA TTACTGATGG TGATTCAGTA
      1170        1180        1190        1200
TTATGATTAT GTCCTCCATT AATTATTCTG TTTCATACAA
      1210        1220        1230        1240
GTCGTGTAAT TTGCTGTTTG TGATTGTACG ATAAATTGAT
      1250        1260        1270        1280
TCAACCTTCT GCGGTGTTGG TTGAAGTTCA AGTAAATTAG
      1290        1300        1310        1320
CTTTATTTAT CATAGTAGCA TTTGATTATT GATGCTCTGT
      1330        1340        1350        1360
AGCTAATGAT AAGCCATTGA AGGGAAGCAG AAATGGTAAA
      1370        1380        1390        1400
GCTTTCTAAA ATGAATCTAC GAATGGATGA TAAAGTTAAT
      1410        1420        1430        1440
GAATATTGTT GATACTTCTG CAATCAGATT ATGAGTTACT
      1450        1460        1470        1480
GAGTCTACTG TTTTTTAAGC CTGTTTCAGA TGATCGATCA
      1490        1500        1510        1520
TCAACAACAA CATATTCAGT GTAGTAGACA TGATCGATCA
      1530        1540        1550        1560
CTTTCTAATT TTCGATTATG CACCCTCTTT TCTCCAATTT
      1570        1580        1590        1600
GGTCGTCTTC TTTTTTTCAT GATGTCACTG AATTATTCTC
      1610        1620        1630        1640
TGGTCGTCCC CACCATTCAG GAAGTCACTT CGAGCATAAT
      1650        1660        1670        1680
GTGAAAACAT CCACATTTTT CAAATCCAGC AGAATTTTCA
```

FIGURE 1 CONTINUED

```
      1690        1700        1710        1720
TCAAACGGGG TTCAACATTT ACTACATGTA TACACTCTGA
      1730        1740        1750        1760
AGTCTGAATC CACTAATTCT AGATGGTGCA TCTGTGCCCC
      1770        1780        1790        1800
CACACTTGTG AAAGCTTATT CTCAATTTTT TATTTTCCAA
      1810        1820        1830        1840
CAACTTGAAT TCAGACCACA CAACTCCCGT GTCTTGTACG
      1850        1860        1870        1880
GTCAGCATCT GAGTGGAGAA CTCAATTAAG TGACTTTAAC
      1890        1900        1910        1920
GTCGAGTTCT ATAGTAAACA ACCCCTATAT CTTTTTTCAA
      1930        1940        1950        1960
GCATGTTAAG ATTGCGAACA CACTGAAATT TCCAGGTCGT
      1970        1980        1990        2000
TAATCTTGTA CCCAGTGTGT GTACTTTTAA AAAAAAAAGT
      2010        2020        2030        2040
CAGTTTTTTA GTCTCTAAAA CACATTTAAA TAGAGTTTAT
      2050        2060        2070        2080
TTGCCATCTT TTGTTCCTCA TACTAGACTT CGGAGTCAAC
      2090
ACAACACAAC AACA
```

FIGURE 2

AMY 351 PROMOTER
SEQUENCE TYPE: Nucleotide
MOLECULE TYPE: DNA (genomic)
ORIGINAL SOURCE: *Solanum tuberosum*
SEQUENCE LENGTH: 1734 bp
STRANDEDNESS: Double
TOPOLOGY: Linear
SEQUENCE:

```
        10         20         30         40
TCTTTAAGTT GTTTGCTTGA TTTTTCTTCT TCAATCTTCT
        50         60         70         80
ATATTTAATT CGTTTTAGCT TCAAACTTCT TCAATTTTAT
        90        100        110        120
TTCAATTTAA TTCTACAAAA AAAATCTCTA TTTAGCACCA
       130        140        150        160
TTCATAAAAT TCATGCTCAA AATGGGCAAA CATAAATAAT
       170        180        190        200
AAATGTGAAG TAAATAATGG ATTAAAATAT ATATTTTTGG
       210        220        230        240
GCCTCACATC AACCTTCATA ATTCTTGAAT GAATGAATGA
       250        260        270        280
TAGACTTCAT AATTTTTTAA CCTATACATA TAAGAAAATT
       290        300        310        320
GAGAGTAACT CAAATAACAA GTTGTAGTAT CACATCTTTA
       330        340        350        360
CTATTTGATA ACATTATGAA GGTGATTATA CATTACGTAA
       370        380        390        400
CATTTCTTTT AAAAATATGT AAGCAAATTT ACTTTTTAAC
       410        420        430        440
TTATCATTGA TCTTCATGGT TTTGTCATAA ATCTCAAAGT
       450        460        470        480
TATCATATTT TATATAGCTA TTTGAAAGTA ATTTTATTTT
       490        500        510        520
TACTCATCAT TGAGTGATGC TTTTATTATA ATACTAGTAA
       530        540        550        560
GTTTTATTTA TTATTTTCTT TTAGGGGTGA ATTGTATAAT
       570        580        590        600
ATAATAAAAA ATATATTTTT AGAAATAATG ATTCTTTTAT
       610        620        630        640
TATTAAAAAG TTAAGATATT AGATTATTTA TGCTTGTATA
       650        660        670        680
ATAATGAACG AAGTTTTATT TTCTATGAGT TTCATTAATC
       690        700        710        720
ATGTTTGTAA TTATTTCAAA TTTTGATGTA TTTTTATAAT
       730        740        750        760
TTTGTATTAT TATATTATTA TACTATATTT AAAAATTTAA
```

FIGURE 2 CONTINUED

```
       770        780        790        800
AGATCCATAG GGCTTACGCC CCACGTCAAG AGGCTTGCGC
       810        820        830        840
CTTTCCCTAA ATTAAGTAAA ACTCTTCGCC TCATGCCTTA
       850        860        870        880
CGCCTCCGCC TTTTAAAACA CTGATTCCTT TCCTCATATA
       890        900        910        920
GCTTGAGGCG AAAATATTTA ATAAAAACAC TTCTTAATTT
       930        940        950        960
GTTTATATGT TCAATTGAAC ATGTCCGTGA TTAGAAAATT
       970        980        990       1000
AAATTAAATT CAATGACAAA TTTAATAATT TGACACAAAA
      1010       1020       1030       1040
TTTATGAAAA AAATATCAAA ATATAAAGAA ATATTTTTTT
      1050       1060       1070       1080
TGAAATGGAT TAAAAAGAAA AAAAAAACAA ATAAATTGAA
      1090       1100       1110       1120
CCGGGATAAG TTGGTTGTTT AATTGATTAT TGATTATGAT
      1130       1140       1150       1160
CTCAATTTGA CATTTTGCGC GATCTTTCGA CCTCAATTCG
      1170       1180       1190       1200
TATGAACTGA CACTACGCCA ATGGACAGTC GCCGTCGTCA
      1210       1220       1230       1240
CCGCCACCGC ACTATTCTCG ACGCGTCGTC TATCTCCTCC
      1250       1260       1270       1280
ACCCCACAGC CGTCAATTCC AAGCTTCCAA TGAACCGTTG
      1290       1300       1310       1320
CCATGTGTCA CTGCCTATTC ACCGCGAAAC ATGAATATCA
      1330       1340       1350       1360
CTGACGAACG ATTTCGGAGC GGAACGAATC CAGAAAATGG
      1370       1380       1390       1400
ATTACTTTCT ATAAATTCCT CGAATCTCAA CTCCATTTCG
      1410       1420       1430       1440
TAAAAATAAA ATTAAAAATA TTGTTTCTTT TTGTATTTCT
      1450       1460       1470       1480
TTTTGTATTT CTGGTTTATG TGGTGATCGA ATTTTCAATT
      1490       1500       1510       1520
TTTTTACTGG TAGTGATTCC TACTTTTCTT CAATTGCATT
      1530       1540       1550       1560
TCTCCTTTTT CCATTTCACG GTTGAGAATT CATGATTCCT
      1570       1580       1590       1600
TATCAGAGGA ATCGATCCGA TTTGACTAAT TTCACTTTTC
      1610       1620       1630       1640
GTCTGTATAA ATACCAGAGT ATCTAGGTTG AGGAACGTAA
      1650       1660       1670       1680
TTTCAAGCTG CGATCGGCTT TTTCCCCTGA ACGAGCAAAC
```

FIGURE 2 CONTINUED

```
      1690        1700        1710        1720
ACAGGTTGTG GGTTCGAGTT AGCAAGGGAC GTATAATCTC
      1730
AACTACAATC CATT
```

FIGURE 3

α-AMYLASE CODING SEQUENCE
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2017 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(A) LENGTH: 475 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

```
ATG AAG TCT CTC GCC GCA ATT GCT GCT CTG CTG TCG CCC ACA CTG GTC        48
Met Lys Ser Leu Ala Ala Ile Ala Ala Leu Leu Ser Pro Thr Leu Val
-18         -15                 -10                 -5

CGG GCA GCG ACT CCG GAT GAG TGG AAA GCT CAG TCG ATC TAT TTC ATG        96
Arg Ala Ala Thr Pro Asp Glu Trp Lys Ala Gln Ser Ile Tyr Phe Met
          1               5                  10

CTG ACG GAC CGG TTT GCG CGT ACC GAC AAT TCG ACC ACG GCT CCC TGT       144
Leu Thr Asp Arg Phe Ala Arg Thr Asp Asn Ser Thr Thr Ala Pro Cys
 15                  20                  25                  30

GAC ACC ACT GCC GGG GTATGCAACT AACCCTGTGT TTCTCTTCCC GGGACGTACA       199
Asp Thr Thr Ala Gly
                 35

AGGGGTCTTC TCCATGCTAA CCGTGCACAT GCAG AAA TAT TGC GGG GGA ACA         251
                                      Lys Tyr Cys Gly Gly Thr
                                                          40

TGG CGA GGT ATC ATC AAC AAC GTAAGTGGCT TCTGATTTTC GCTCAATAAT         302
Trp Arg Gly Ile Ile Asn Asn
             45

CTTCGTCGCG TGACTTTATT TCCTAG CTG GAT TAC ATC CAG GAT ATG GGC TTC      355
                             Leu Asp Tyr Ile Gln Asp Met Gly Phe
                                 50                  55

ACA GCT ATC TGG ATA ACT CCA GTG ACA GCC CAG TGG GAC GAC GAT GTG       403
uThr Ala Ile Trp Ile Thr Pro Val Thr Ala Gln Trp Asp Asp Asp Val
        60                  65                  70

GAT GCG GCA GAT GCA ACG TCG TAT CAC GGT TAT TGG CAG AAA GAC  CT       450
Asp Ala Ala Asp Ala Thr Ser Tyr His Gly Tyr Trp Gln Lys Asp  Leu
     75                  80                  85

GTGCGCAACC CTGCTCCATG GATCGCTGGC TGCAAACTCG TGCTGATCGG TGATTTTTTT     510

TTTTTTTTTT TTGAAACAG A TAC TCT CTG AAT TCG AAA TTC GGC ACT GCC       560
                      Tyr Ser Leu Asn Ser Lys Phe Gly Thr Ala
                       90                  95
```

FIGURE 3 CONTINUED

```
GAT GAC TTG AAA GCC CTG GCT GAT GCC CTT CAC GCC CGT GGG ATG CTT          608
Asp Asp Leu Lys Ala Leu Ala Asp Ala Leu His Ala Arg Gly Met Leu
100                 105                 110                 115

CTC ATG GTC GAC GTC GTG GCT AAT CAC TTT GTACGGACCA TCTACATACC           658
Leu Met Val Asp Val Val Ala Asn His Phe
                120                 125

TGGGAAACGC GAAGAAGGAA AAAAAAAAAA AGGCGCACGC TAACATTTCG CGTTTAG          715

GGC TAC GGC GGT TCT CAT AGC GAG GTG GAT TAC TCG ATC TTC AAT CCT          763
Gly Tyr Gly Gly Ser His Ser Glu Val Asp Tyr Ser Ile Phe Asn Pro
                130                 135                 140

CTG AAC AGC CAG GAT TAC TTC CAC CCG TTC TGT CTC ATT GAG GAC TAC          811
Leu Asn Ser Gln Asp Tyr Phe His Pro Phe Cys Leu Ile Glu Asp Tyr
            145                 150                 155

GAC AAC CAG GAA GAA GTC GAA CAA TGC TGG CTG GCC GAT ACT CCG ACG          859
Asp Asn Gln Glu Glu Val Glu Gln Cys Trp Leu Ala Asp Thr Pro Thr
        160                 165                 170

ACA TTG CCC GAC GTG GAC ACC ACC AAT CCT CAG GTT CGG ACG TTT TTC          907
Thr Leu Pro Asp Val Asp Thr Thr Asn Pro Gln Val Arg Thr Phe Phe
    175                 180                 185

AAC GAC TGG ATC AAG AGC CTG GTG GCG AAC TAC TCC  A GTATGATTGT           954
Asn Asp Trp Ile Lys Ser Leu Val Ala Asn Tyr Ser
190                 195                 200

TCCCGCGGTA ACGCTTTAGG GCTTGCTCTA ACTGAAATCG ACAG  TC GAT GGT CTG        1009
                                                 Ile Asp Gly Leu
                                                             205

CGC GTC GAC ACC GTT AAG CAC GTG GAG AAA GAT TTC TGG CCC GAC TTC         1057
Arg Val Asp Thr Val Lys His Val Glu Lys Asp Phe Trp Pro Asp Phe
                210                 215                 220

AAC GAA GCT GCT GCG TGT ACC GTC GGC GAG GTG TTC AAC GGT GAC CCA         1105
Asn Glu Ala Ala Ala Cys Thr Val Gly Glu Val Phe Asn Gly Asp Pro
            225                 230                 235

GCG TAC ACC TGC CCA TAC CAG GAA GTG CTG GAT GGC GTT CTG AAC TAT         1153
Ala Tyr Thr Cys Pro Tyr Gln Glu Val Leu Asp Gly Val Leu Asn Tyr
        240                 245                 250

CCG  AT  GTGAGTGATT CCGAAAGTTC CATCGATCAG GCTTTCTGAC GCATGAGAAC         1208
Pro  Ile
     255
```

FIGURE 3 CONTINUED

```
AGC TAC TAT CCT GCG CTT GAT GCA TTC AAG TCT GTC GGC GGC AAT CTC      1256
    Tyr Tyr Pro Ala Leu Asp Ala Phe Lys Ser Val Gly Gly Asn Leu
                    260                 265                 270

GGC GGC TTG GCT CAG GCC ATC ACC ACC GTG CAG GAG AGC TGC AAG GAT      1304
Gly Gly Leu Ala Gln Ala Ile Thr Thr Val Gln Glu Ser Cys Lys Asp
                275                 280                 285

TCC AAT CTG CTC GGC AAT TTC CTT GAG AAT CAC GAC ATT GCT CGC TTT      1352
Ser Asn Leu Leu Gly Asn Phe Leu Glu Asn His Asp Ile Ala Arg Phe
            290                 295                 300

GCT  TC  GTATGGACAC TCTTTTTGAA GCCCTCATCG ATTGGGGATG CTGACACGGA      1407
Ala  Ser

CAACAACAAC AG G TAC ACG GAT GAC CTT GCT CTC GCC AAG AAT GGT CTC      1456
              Tyr Thr Asp Asp Leu Ala Leu Ala Lys Asn Gly Leu
              305                 310                 315

GCT TTC ATC ATC CTC TCG GAT GGT ATT CCG ATC ATC TAC ACG GGC CAG      1504
Ala Phe Ile Ile Leu Ser Asp Gly Ile Pro Ile Ile Tyr Thr Gly Gln
            320                 325                 330

GAG CAG CAC TAC GCC GGT GAT CAC GAT CCC ACA AAT CGT GAG GCC GTC      1552
Glu Gln His Tyr Ala Gly Asp His Asp Pro Thr Asn Arg Glu Ala Val
        335                 340                 345

TGG CTG TCT GGC TAC AAT ACC GAC GCC GAG CTG TAC CAG TTC ATC AAG      1600
Trp Leu Ser Gly Tyr Asn Thr Asp Ala Glu Leu Tyr Gln Phe Ile Lys
    350                 355                 360

AAG GCC AAT GGC ATC CGC AAC TTG GCT ATC AGC CAG AAC CCG GAA TTC      1648
Lys Ala Asn Gly Ile Arg Asn Leu Ala Ile Ser Gln Asn Pro Glu Phe
365                 370                 375                 380

ACC TCC TCC AAG GTGAGTACAA TAACAAACTT TTCGAAAAAT TTTTCACCGG          1700
Thr Ser Ser Lys

AGAAAACCTA AGATTCGGCT AACAAAACAA AAAAAAAAAA G ACC AAG GTC ATC        1753
                                              Thr Lys Val Ile
                                              385

TAC CAA GAC GAT TCG ACC CTT GCC ATT AAC CGG GGC GGC GTC GTT ACT      1801
Tyr Gln Asp Asp Ser Thr Leu Ala Ile Asn Arg Gly Gly Val Val Thr
    390                 395                 400

GTC CTG AGC AAT GAA GGC GCC TCC GGG GAG ACC GGG ACT GTC TCC ATT      1849
Val Leu Ser Asn Glu Gly Ala Ser Gly Glu Thr Gly Thr Val Ser Ile
405                 410                 415                 420
```

FIGURE 3 CONTINUED

```
CCG GGA ACT GGC TTC GAG GCC GGC ACG GAA TTG ACT GAT GTC ATC TCC      1897
Pro Gly Thr Gly Phe Glu Ala Gly Thr Glu Leu Thr Asp Val Ile Ser
                425                 430                 435

TGC AAG ACC GTG ACT GCG GGG GAC AGC GGG GCG GTC GAC GTG CCC TTG      1945
Cys Lys Thr Val Thr Ala Gly Asp Ser Gly Ala Val Asp Val Pro Leu
            440                 445                 450

TCG GGC GGA CTG CCA AGC GTG CTC TAT CCC AGC TCC CAG CTG GCC AAG      1993
Ser Gly Gly Leu Pro Ser Val Leu Tyr Pro Ser Ser Gln Leu Ala Lys
        455                 460                 465

AGT GGT CTG TGT GCG TCG GCG TGA                                      2017
Ser Gly Leu Cys Ala Ser Ala
    470                 475
```

FIGURE 1

α-AMYLASE CODING SEQUENCE
SEQUENCE TYPE: Nucleotide
MOLECULE TYPE: DNA
ORIGINAL SOURCE: *Solanum Tuberosum*
SEQUENCE LENGTH: 1570
SEQUENCE:

```
        10         20         30         40
TGTGGTGATC GAATTTTCAA TTTTTTACT GAGTATCTAG
        50         60         70         80
GTTGAGGAAC GTAATTTCAA GCTGCGATCG GCTTTTTCCC
        90        100        110        120
CTGAACGAGC AAACACAGGT TGTGGGTTCG AGTTAGCAAG
       130        140        150        160
GGACGTATAA TCTCAACTAC AATCCATTAT GGCGCTTGAT
       170        180        190        200
GAAAGTCAGC AGTCTGATCC ATTGGTTGTG ATACGCAATG
       210        220        230        240
GAAAGGAGAT CATATTGCAG GCATTCGACT GGGAATCTCA
       250        260        270        280
TAAACATGAT TGGTGGCTAA ATTTAGATAC GAAAGTTCCT
       290        300        310        320
GATATTGCAA AGTCTGGTTT CACAACTGCT TGGCTGCCTC
       330        340        350        360
CGGTGTGTCA GTCATTGGCT CCTGAAGGTT ACCTTCCACA
       370        380        390        400
GAACCTTTAT TCTCTCAATT CTAAATATGG TTCTGAGGAT
       410        420        430        440
CTCTTAAAAG CTTTACTTAA TAAGATGAAG CAGTACAAAG
       450        460        470        480
TTAGAGCGAT GGCGGACATA GTCATTAACC ACCGTGTTGG
       490        500        510        520
GACTACTCAA GGGCATGGTG GAATGTACAA CCGCTATGAT
       530        540        550        560
GGAATTCCTA TGTCTTGGGA TGAACATGCT ATTACATCTT
       570        580        590        600
GCACTGGTGG AAGGGGTAAC AAAAGCACTG GAGACAACTT
       610        620        630        640
TAATGGAGTT CCAAATATAG ATCATACACA ATCCTTTGTT
       650        660        670        680
CGGAAAGATC TCATTGACTG GATGCGGTGG CTAAGATCCT
       690        700        710        720
CTGTTGGCTT CCAAGATTTT CGTTTTGATT TTGCCAAAGG
       730        740        750        760
TTATGCTTCA AAGTATGTAA AGGAATATAT CGAGGGAGCT
       770        780        790        800
GAGCCAATAT TTGCAGTTGG AGAATACTGG GACACTTGCA
       810        820        830        840
ATTACAAGGG CAGCAATTTG GATTACAACC AAGATAGTCA
       850        860        870        880
CAGGCAAAGA ATCATCAATT GGATTGATGG CGCGGGACAA
       890        900        910        920
CTTTCAACTG CATTCGATTT TACAACAAAA GCAGTCCTTC
```

FIGURE 4 CONTINUED

```
       930         940         950         960
AGGAAGCAGT CAAAGGAGAA TTCTGGCGTT TGCGTGACTC
       970         980         990        1000
TAAGGGGAAG CCCCCAGGAG TTTTAGGATT GTGGCCTTCA
      1010        1020        1030        1040
AGGGCTGTCA CTTTTATTGA TAATCACGAC ACTGGATCAA
      1050        1060        1070        1080
CTCAGGCGCA TTGGCCTTTC CGTTCACGTC ATGTTATGGA
      1090        1100        1110        1120
GGGCTATGCA TACATTCTTA CACACCCAGG GATACCATCA
      1130        1140        1150        1160
GTTTTCTTTG ACCATTTCTA CGAATGGGAT AATTCCATGC
      1170        1180        1190        1200
ATGACCAAAT TGTAAAGCTG ATTGCTATTC GGAGGAATCA
      1210        1220        1230        1240
AGGCATACAC AGCCGTTCAT CTATAAGAAT TCTTGAGGCA
      1250        1260        1270        1280
CAGCCAAACT TATACGCTGC AACCATTGAT GAAAAGGTTA
      1290        1300        1310        1320
GCGTGAAGAT TGGGGACGGA TCATGGAGCC CTGCTGGGAA
      1330        1340        1350        1360
AGAGTGGACT CTCGCGACCA GTGGCCATCG CTATGCAGTC
      1370        1380        1390        1400
TGGCAGAAGT AATCTTACAG CTATTCCGTT ACTTAATATA
      1410        1420        1430        1440
TTAGTAGAAA TATATATGTT TTAAACCCGA GCACCTACTT
      1450        1460        1470        1480
CTAACACTAG ATCCGCCTCT ACAGGCTTGG ATGGAGTGAT
      1490        1500        1510        1520
GAGTTTTTTT TTCCTGTTCA TTAGACATTG CAACATGGGA
      1530        1540        1550        1560
TGTATGTTTT GTTAATAAAA GTGTTCTTGA TCAATGCAAT
      1570
GTAATAAGGG
```

FIGURE 5

SEQUENCE: Nucleotide sequence of a cDNA encoding the large subunit of ADP-glucose pyrophosphorylase from barley seed endosperm (bepl10)
SEQUENCE TYPE: NUCLEIC ACID
MOLECULE TYPE: DNA
ORIGINAL SOURCE: BARLEY
SEQUENCE LENGTH: 2037
STRANDEDNESS: DOUBLE
TOPOLOGY: LINEAR

```
1     ACGACCACCT CCGAACTCAA CGCCTCCACG GACCATCTCT
41    CTCCTCTCCC CTCCCCTCAC CACCACCACC ACCACCACCC
81    CTTCTCCCTC CCTGCATTTG ATTCGTTCAT ATTCATCCGT
121   CGCTTGCCCG GTCGCCACCC CGTCGATCCC TCACCCCGCC
161   GTCCCCGGCA GTTGCAGGTG GACTGCTAAT GTCATCGATG
201   CAGTTCAGCA GCGTGCTGCC CCTGGAGGGC AAGGCGTGCG
241   TTTCCCCAGT CAGGAGAGAG GGATCGGCCT GCGAGCGCCT
281   CAAGATCGGG GACAGCAGCA GCATCAGGCA CGAGAGAGCG
321   TCCAGGAGGA TGTGCAACGG CGGCGCAGGG GCCCCGCCGC
361   CACCGGTGCG CAGTGCGTGC TCACCTCCGA CGCCAGCCCG
401   GCCGACACCC TTGTTCTCCG GACGTCCTTC CGGAGGAATT
      ACGCCGATCC GAACGAGGTC GCGGCCGTCG GTCGCGGCCG
      TCATACTCGG CGGCGGCACC GGGACTCAGC TCTTCCCGCT
      CACAAGCACA AGGGCCACAC CTGCTGTTCC TATTGGAGGA
      TGTTACAGGC TCATCGATAT TCCCATGAGC AACTGCTTCA
601   ACAGTGGCAT CAACAAGATA TTCGTCATGA CCCAGTTCAA
      CTCGGCATCT CTCAATCGCC ACATTCACCG CACCTACCTC
      GGCGGGGGAA TCAATTTCAC TGATGGATCT GTTGAGGTAT
      TGGCCGCGAC ACAAATGCCT GGGGAGGCTG CTGGATGGTT
      CCGCGGAACA GCGGATGCCG TCAGAAAATT TATCTGGGTG
801   CTTGAGGACT ACTATAAGCA TAAATCCATA GAGCACATTT
      TGATCTTGTC GGGCGATCAG CTTTATCGCA TGGATTACAT
      GGAGCTTGTG CAGAAACATG TGGATGACAA TGCTGACATT
      ACTTTATCAT GTGCCCCTGT TGGAGAGAGC CGGGCATCTG
      AGTACGGGCT AGTGAAGTTC GACAGTTCAG GCCGTGTGAT
1001  CCAGTTTTCT GAGAAGCCAA AGGGCGACGA TCTGGAAGCG
      ATGAAAGTGG ATACCAGTTT TCTCAATTTC GCCATAGACG
      ACCCTGCTAA ATATCCATAC ATTGCTTCGA TGGGAGTTTA
      TGTCTTCAAG AGAGATGTTC TGCTGAACCT TCTAAAGTCA
      AGATACGCAG AACTACATGA CTTTGGGTCT GAAATCCTCC
1201  CGAGAGCTCT GCATGATCAC AATGTACAGG CATATGTCTT
      CACTGACTAC TGGGAGGACA TTGGAACAAT CAGATCCTTC
      TTCGATGCGA ACATGGCCCT CTGCGAACAG CCTCCAAAGT
      TTGAATTTTA TGATCCAAAA ACCCCCTTCT TCACTTCGCC
      TCGGTACTTA CCGCCAACAA AGTCAGACAA GTGCAGGATC
1401  AAAGAAGCGA TCATTTCGCA CGGCTGCTTC TTGCGTGAAT
      GCAAAATCGA GCACTCCATC ATCGGCGTTC GTTCACGCCT
      AAACTCCGGA AGCGAGCTCA AGAACGCGAT GATGATGGGC
      GCGGACTCGT ACGAGACCGA GGACGAGATC TCGAGGCTGA
      TGTCTGAGGG CAAGGTTCCC ATCGGCGTCG GGGAGAACAC
1601  AAAGATCAGC AACTGCATCA TCGACATGAA CGCGAGGATA
```

FIGURE 5 CONTINUED

```
      GGAAGGGACG TGGTCATCTC AAACAAGGAG GGGGTGCAAG
      AAGCCGACAG GCCGGAGGAA GGGTACTACA TCAGGTCCGG
      GATCGTGGTG ATCCAGAAGA ACGCGACCAT CAAGGACGGC
      ACCGTCGTGT AGGGCGTGCC GGGTCGGCGC GACGGGGTTC
1801  TGCGACAACC TGTGCGCTGC GTCGGTCGTC ATCATCTTCT
      CAAACTCCGG GACTGAAGAA GTGATCCGGG GACGGGAGAC
      GTTTGAAGCT TGAATGACTG AGACTGAAAG TGAAGGCGCA
      GCAGAGGCAG GCAGCATTAG TAGTAAGTAG TAAGTAAGTA
      GCAGTGGAAC AAAGTAATAG TCGTTCGTTT TTCCCCTGTA
2001  ATAAATAAGA GGCTGTGTGT TGAGGTAAAA AAAAAAA
```

FIGURE 6

SEQUENCE: Nucleotide sequence of a cDNA encoding the small subunit of ADP-glucose pyrophosphorylase from barley seed endosperm (bepS)
SEQUENCE TYPE: NUCLEIC ACID
MOLECULE TYPE: DNA
ORIGINAL SOURCE: BARLEY
SEQUENCE LENGTH: 1822
STRANDEDNESS: DOUBLE
TOPOLOGY: LINEAR
COMMENT: The "." at 1569 denotes a purine.

```
1     AAAAGTGAAC TCACACATCA CTCAATATCT ATATCCTTCC
      ATTTTATATC CCTCGGTGAT GGATGTACCT TTGGCATCTA
      AAGTTCCCTT GCCCTCCCCT TCCAAGCATG AACAATGCAA
      CGTTTATAGT CATAAGAGCT CATCGAAGCA TGCAGATCTC
      AATCCCCATG CTATTGATAG TGTTCTCGGT ATCATTCTTG
201   GAGGTGGTGC AGGGACTAGA TTGTATCCCC TGACGAAGAA
      GCGTGCAAAG CCTGCAGTGC CATTGGGTGC CAACTACAGG
      CTTATTGATA TTCCTGTCAG TAATTGTCTG AACAGCAACA
      TATCAAAGAT CTATGTGCTT ACACAGTTCA ACTCAGCTTC
      TCTTAATCGT CATCTCTCAC GAGCCTATGG GAGCAACATT
401   GGAGGTTACA AGAATGAAGG ATTTGTTGAA GTCCTTGCTG
      CACAGCAGAG CCCAGATAAC CCTGACTGGT TCCAGGGTAC
      TGCAGATGCT GTAAGGCAGT ACTTGTGGCT ATTCGAGGAG
      CATAATGTTA TGGAGTATCT AATTCTTGCT GGAGATCACC
      TGTACCGAAT GGACTATGAA AAGTTTATTC AGGCACACAG
601   AGAAACGGAT GCTGATATTA CTGTTGCTGC CTTGCCCATG
      GATGAGGAAC GTGCAACTGC ATTTGGCCTT ATGAAAATCG
      ATGAAGAAGG GAGGATAATT GAATTCGCAG AGAAACCAAA
      AGGAGAACAG TTGAAAGCTA TGATGGTTGA TACGACCATA
      CTTGGCCTTG AAGATGCGAG GGCAAAGGAA ATGCCTTATA
801   TTGCTAGCAT GGGTATCTAT GTTATTAGCA AACATGTGAT
      GCTTCAGCTT CTCCGTGAGC AATTTCCTGG AGCTAATGAC
      TTCGGAAGTG AAGTTATCCC TGGTGCAACT AGCACTGGCA
      TGAGGGTACA AGCATACCTA TACGACGGTT ACTGGGAAGA
      TATTGGTACA ATTGAGGCAT TCTATAATGC AAATTTGGGA
1001  ATTACCAAAA AACCAATACC TGATTTCAGT TTCTATGACC
      GTTCTGCTCC CATTTACACA CAACCTCGAC ACTTGCCTCC
      TTCAAAGGTT CTTGATGCTG ATGTGACAGA CAGTGTAATT
      GGTGAAGGAT GTGTTATTAA AAACTGCAAG ATACACCATT
      CAGTAGTTGG ACTCCGTTCC TGCATATCTG AAGGTGCAAT
1201  AATAGAGGAC ACGTTGCTAA TGGGTGCGGA CTACTATGAG
      ACTGAAGCTG ATAAGAAACT CCTTGCTGAA AAAGGTGGCA
      TTCCCATTGG TATTGGAAAG AATTCACACA TCAAAAGAGC
      AATCATTGAC AAGAATGCTC GTATTGGAGA TAACGTGATG
      ATAATCAATG TTGACAATGT TCAAGAAGCG GCGAGGGAGA
1401  CAGATGGATA CTTCATCAAA AGTGGCATCG TAACTGTGAT
      CAAGGATGCT TTACTCCCTA GTGGAACAGT CATATGAAGC
      AGATGTGAAA TGTATGCCAA AAGACAGGGC TACTTGCGTC
      AGTCTGGAAT CAACCAACAA GGCCGCGAAG GAGATCATAA
      AATAAAAA.G GAGTGCCATG CGAGTCACTT CTACACCCTT
1601  TTCCCCCCTT GATGTATTAG GAACTGTGAT GTACAAGCAA
```

FIGURE 6 CONTINUED

```
      CTGTGATGCA CTTACGCGAA GTGCCCCTGG ATTCAGCTTT
      CTCTTTGCTT GTAACTGGTT TCCAGCAGAC CATGCTATTT
      GTTGTATGGT TCGTGCAAAA CCTTGCGATG CTTTATATAT
      GCTTTATATA TAAACAAGAT GAATCCCCGC GCGTTGCTGC
2001  GGCACAAAAA AAAAAAAAAA AA
```

FIGURE 7

α-GLUCAN LYASE CODING SEQUENCE
SEQUENCE TYPE: NUCLEIC ACID
MOLECULE TYPE: DNA (GENOMIC)
ORIGINAL SOURCE: FUNGALLY INFECTED ALGAE
SEQUENCE LENGTH: 3267 BP
STRANDEDNESS: DOUBLE
SEQUENCE:

```
                10         20         30         40         50         60
                 |          |          |          |          |
   1 ATGTTTTCAA CCCTTGCGTT TGTCGCACCT AGTGCGCTGG GAGCCAGTAC CTTCGTAGGG
  61 GCGGAGGTCA GGTCAAATGT TCGTATCCAT TCCGCTTTTC CAGCTGTGCA CACAGCTACT
 121 CGCAAAACCA ATCGCCTCAA TGTATCCATG ACCGCATTGT CCGACAAACA AACGGCTACT
 181 GCGGGTAGTA CAGACAATCC GGACGGTATC GACTACAAGA CCTACGATTA CGTCGGAGTA
 241 TGGGGTTTCA GCCCCCTCTC CAACACGAAC TGGTTTGCTG CCGGCTCTTC TACCCCGGGT
 301 GGCATCACTG ATTGGACGGC TACAATGAAT GTCAACTTCG ACCGTATCGA CAATCCGTCC
 361 ATCACTGTCC AGCATCCCGT TCAGGTTCAG GTCACGTCAT ACAACAACAA CAGCTACAGG
 421 GTTCGCTTCA ACCCTGATGG CCCTATTCGT GATGTGACTC GTGGGCCTAT CCTCAAGCAG
 481 CAACTAGATT GGATTCGAAC GCAGGAGCTG TCAGAGGGAT GTGATCCCGG AATGACTTTC
 541 ACATCAGAAG GTTTCTTGAC TTTTGAGACC AAGGATCTAA GCGTCATCAT CTACGGAAAT
 601 TTCAAGACCA GAGTTACGAG AAAGTCTGAC GGCAAGGTCA TCATGGAAAA TGATGAAGTT
 661 GGAACTGCAT CGTCCGGGAA CAAGTGCCGG GGATTGATGT TCGTTGATAG ATTATACGGT
 721 AACGCTATCG CTTCCGTCAA CAAGAACTTC CGCAACGACG CGGTCAAGCA GGAGGGATTC
 781 TATGGTGCAG GTGAAGTCAA CTGTAAGTAC CAGGACACCT ACATCTTAGA ACGCACTGGA
 841 ATCGCCATGA CAAATTACAA CTACGATAAC TTGAACTATA ACCAGTGGGA CCTTAGACCT
 901 CCGCATCATG ATGGTGCCCT CAACCCAGAC TATTATATTC CAATGTACTA CGCAGCACCT
 961 TGGTTGATCG TTAATGGATG CGCCGGTACT TCGGAGCAGT ACTCGTATGG ATGGTTCATG
1021 GACAATGTCT CTCAATCTTA CATGAATACT GGAGATACTA CCTGGAATTC TGGACAAGAG
1081 GACCTGGCAT ACATGGGCGC GCAGTATGGA CCATTTGACC AACATTTTGT TTACGGTGCT
1141 GGGGGTGGGA TGGAATGTGT GGTCACAGCG TTCTCTCTTC TACAAGGCAA GGAGTTCGAG
1201 AACCAAGTTC TCAACAAACG TTCAGTAATG CCTCCGAAAT ACGTCTTTGG TTTCTTCCAG
1261 GGTGTTTTCG GGACTTCTTC CTTGTTGAGA GCGCATATGC CAGCAGGTGA GAACAACATC
1321 TCAGTCGAAG AAATTGTAGA AGGTTATCAA AACAACAATT TCCCTTTCGA GGGGCTCGCT
1381 GTGGACGTGG ATATGCAAGA CAACTTGCGG GTGTTCACCA CGAAGGGCGA ATTTTGGACC
1441 GCAAACAGGG TGGGTACTGG CGGGGATCCA AACAACCGAT CGGTTTTTGA ATGGGCACAT
1501 GACAAAGGCC TTGTTTGTCA GACAAATATA ACTTGCTTCC TGAGGAATGA TAACGAGGGG
1561 CAAGACTACG AGGTCAATCA GACGTTAAGG GAGAGGCAGT TGTACACGAA GAACGACTCC
1621 CTGACGGGTA CGGATTTTGG AATGACCGAC GACGGCCCCA GCGATGCGTA CATCGGTCAT
1681 CTGGACTATG GGGGTGGAGT AGAATGTGAT GCACTTTTCC CAGACTGGGG ACGGCCTGAC
1741 GTGGCCGAAT GGTGGGGAAA TAACTATAAG AAACTGTTCA GCATTGGTCT CGACTTCGTC
1801 TGGCAAGACA TGACTGTTCC AGCAATGATG CCGCACAAAA TTGGCGATGA CATCAATGTG
1861 AAACCGGATG GGAATTGGCC GAATGCGGAC GATCCGTCCA ATGGACAATA CAACTGGAAG
1921 ACGTACCATC CCCAAGTGCT TGTAACTGAT ATGCGTTATG AGAATCATGG TCGGGAACCG
1981 ATGGTCACTC AACGCAACAT TCATGCGTAT ACACTGTGCG AGTCTACTAG GAAGGAAGGG
2041 ATCGTGGAAA ACGCAGACAC TCTAACGAAG TTCCGCCGTA GCTACATTAT CAGTCGTGGT
2101 GGTTACATTG GTAACCAGCA TTTCGGGGGT ATGTGGGTGG GAGACAACTC TACTACATCA
2161 AACTACATCC AAATGATGAT TGCCAACAAT ATTAACATGA ATATGTCTTG CTTGCCTCTC
2221 GTCGGCTCCG ACATTGGAGG ATTCACCTCA TACGACAATG AGAATCAGCG AACGCCGTGT
2281 ACCGGGGACT TGATGGTGAG GTATGTGCAG GCGGGCTGCC TGTTGCCGTG GTTCAGGAAC
2341 CACTATGATA GGTGGATCGA GTCCAAGGAC CACGGAAAGG ACTACCAGGA GCTGTACATG
2401 TATCCGAATG AAATGGATAC GTTGAGGAAG TTCGTTGAAT TCCGTTATCG CTGGCAGGAA
2461 GTGTTGTACA CGGCCATGTA CCAGAATGCG GCTTTCGGAA AGCCGATTAT CAAGGCTGCT
2521 TCGATGTACA ATAACGACTC AAACGTTCGC AGGGCGCAGA ACGATCATTT CCTTCTTGGT
2581 GGACATGATG GATATCGCAT TCTGTGCGCG CCTGTTGTGT GGGAGAATTC GACCGAACGC
```

FIGURE 7 CONTINUED

2641 GAATTGTACT TGCCCGTGCT GACCCAATGG TACAAATTCG GTCCCGACTT TGACACCAAG
2701 CCTCTGGAAG GAGCGATGAA CGGAGGGGAC CGAATTTACA ACTACCCTGT ACCGCAAAGT
2761 GAATCACCAA TCTTCGTGAG AGAAGGTGCG ATTCTCCCTA CCCGCTACAC GTTGAACGGT
2821 GAAAACAAAT CATTGAACAC GTACACGGAC GAAGATCCGT TGGTGTTTGA AGTATTCCCC
2881 CTCGGAAACA ACCGTGCCGA CGGTATGTGT TATCTTGATG ATGGCGGTGT GACCACCAAT
2941 GCTGAAGACA ATGGCAAGTT CTCTGTCGTC AAGGTGGCAG CGGAGCAGGA TGGTGGTACG
3001 GAGACGATAA CGTTTACGAA TGATTGCTAT GAGTACGTTT TCGGTGGACC GTTCTACGTT
3061 CGAGTGCGCG GCGCTCAGTC GCCGTCGAAC ATCCACGTGT CTTCTGGAGC GGGTTCTCAG
3121 GACATGAAGG TGAGCTCTGC CACTTCCAGG GCTGCGCTGT TCAATGACGG GGAGAACGGT
3181 GATTTCTGGG TTGACCAGGA GACAGATTCT CTGTGGCTGA AGTTGCCCAA CGTTGTTCTC
3241 CCGGACGCTG TGATCACAAT TACCTAA

FIGURE 8

α-GLUCAN LYASE CODING SEQUENCE
SEQUENCE TYPE: NUCLEIC ACID
MOLECULE TYPE: DNA (GENOMIC)
ORIGINAL SOURCE: FUNGALLY INFECTED ALGAE
SEQUENCE LENGTH: 3276 BP
STRANDEDNESS: DOUBLE
SEQUENCE:

```
                10         20         30         40         50         60
                 |          |          |          |          |          |
   1 ATGTATCCAA CCCTCACCTT CGTGGCGCCT AGTGCGCTAG GGGCCAGAAC TTTCACGTGT
  61 GTGGGCATTT TTAGGTCACA CATTCTTATT CATTCGGTTG TTCCAGCGGT GCGTCTAGCT
 121 GTGCGCAAAA GCAACCGCCT CAATGTATCC ATGTCCGCTT TGTTCGACAA ACCGACTGCT
 181 GTTACTGGAG GGAAGGACAA CCCGGACAAT ATCAATTACA CCACTTATGA CTACGTCCCT
 241 GTGTGGCGCT TCGACCCCCT CAGCAATACG AACTGGTTTG CTGCCGGATC TTCCACTCCC
 301 GGCGATATTG ACGACTGGAC GGCGACAATG AATGTGAACT TCGACCGTAT CGACAATCCA
 361 TCCTTCACTC TCGAGAAACC GGTTCAGGTT CAGGTCACGT CATACAAGAA CAATTGTTTC
 421 AGGGTTCGCT TCAACCCTGA TGGTCCTATT CGCGATGTGG ATCGTGGGCC TATCCTCCAG
 481 CAGCAACTAA ATTGGATCCG GAAGCAGGAG CAGTCGAAGG GGTTTGATCC TAAGATGGGC
 541 TTCACAAAAG AAGGTTTCTT GAAATTTGAG ACCAAGGATC TGAACGTTAT CATATATGGC
 601 AATTTTAAGA CTAGAGTTAC GAGGAAGAGG GATGGAAAAG GGATCATGGA GAATAATGAA
 661 GTGCCGGCAG GATCGTTAGG GAACAAGTGC CGGGGATTGA TGTTTGTCGA CAGGTTGTAC
 721 GGCACTGCCA TCGCTTCCGT TAATGAAAAT TACCGCAACG ATCCCGACAG GAAAGAGGGG
 781 TTCTATGGTG CAGGAGAAGT AAACTGCGAG TTTTGGGACT CCGAACAAAA CAGGAACAAG
 841 TACATCTTAG AACGAACTGG AATCGCCATG ACAAATTACA ATTATGACAA CTATAACTAC
 901 AACCAGTCAG ATCTTATTGC TCCAGGATAT CCTTCCGACC CGAACTTCTA CATTCCCATG
 961 TATTTTGCAG CACCTTGGGT AGTTGTTAAG GGATGCAGTG GCAACAGCGA TGAACAGTAC
1021 TCGTACGGAT GGTTTATGGA TAATGTCTCC CAAACTTACA TGAATACTGG TGGTACTTCC
1081 TGGAACTGTG GAGAGGAGAA CTTGGCATAC ATGGGAGCAC AGTGCGGTCC ATTTGACCAA
1141 CATTTTGTGT ATGGTGATGG AGATGGTCTT GAGGATGTTG TCCAAGCGTT CTCTCTTCTG
1201 CAAGGCAAAG AGTTTGAGAA CCAAGTTCTG AACAAACGTG CCGTAATGCC TCCGAAATAT
1261 GTGTTTGGTT ACTTTCAGGG AGTCTTTGGG ATTGCTTCCT TGTTGAGAGA GCAAAGACCA
1321 GAGGGTGGTA ATAACATCTC TGTTCAAGAG ATTGTCGAAG GTTACCAAAG CAATAACTTC
1381 CCTTTAGAGG GGTTAGCCGT AGATGTGGAT ATGCAACAAG ATTTGCGCGT GTTCACCACG
1441 AAGATTGAAT TTTGGACGGC AAATAAGGTA GGCACCGGGG GAGACTCGAA TAACAAGTCG
1501 GTGTTTGAAT GGGCACATGA CAAAGGCCTT GTATGTCAGA CGAATGTTAC TTGCTTCTTG
1561 AGAAACGACA ACGGCGGGGC AGATTACGAA GTCAATCAGA CATTGAGGGA GAAGGGTTTG
1621 TACACGAAGA ATGACTCACT GACGAACACT AACTTCGGAA CTACCAACGA CGGGCCGAGC
1681 GATGCGTACA TTGGACATCT GGACTATGGT GGCGGAGGGA ATTGTGATGC ACTTTTCCCA
1741 GACTGGGGTC GACCGGGTGT GGCTGAATGG TGGGGTGATA ACTACAGCAA GCTCTTCAAA
1801 ATTGGTCTGG ATTTCGTCTG GCAAGACATG ACAGTTCCAG CTATGATGCC ACACAAAGTT
1861 GGCGACGCAG TCGATACGAG ATCACCTTAC GGCTGGCCGA ATGAGAATGA TCCTTCGAAC
1921 GGACGATACA ATTGGAAATC TTACCATCCA CAAGTTCTCG TAACTGATAT GCGATATGAG
1981 AATCATGGAA GGGAACCGAT GTTCACTCAA CGCAATATGC ATGCGTACAC ACTCTGTGAA
2041 TCTACGAGGA AGGAAGGGAT TGTTGCAAAT GCAGACACTC TAACGAAGTT CCGCCGCAGT
2101 TATATTATCA GTCGTGGAGG TTACATTGGC AACCAGCATT TTGGAGGAAT GTGGGTTGGA
2161 GACAACTCTT CCTCCCAAAG ATACCTCCAA ATGATGATCG CGAACATCGT CAACATGAAC
2221 ATGTCTTGCC TTCCACTAGT TGGGTCCGAC ATTGGAGGTT TTACTTCGTA TGATGGACGA
2281 AACGTGTGTC CCGGGGATCT AATGGTAAGA TTCGTGCAGG CGGGTTGCTT ACTACCGTGG
2341 TTCAGAAACC ACTATGGTAG GTTGGTCGAG GGCAAGCAAG AGGGAAAATA CTATCAAGAA
2401 CTGTACATGT ACAAGGACGA GATGGCTACA TTGAGAAAAT TCATTGAATT CCGTTACCGC
2461 TGGCAGGAGG TGTTGTACAC TGCTATGTAC CAGAATGCGG CTTTCGGGAA ACCGATTATC
2521 AAGGCAGCTT CCATGTACGA CAACGACAGA AACGTTCGCG GCGCACAGGA TGACCACTTC
2581 CTTCTCGGCG GACACGATGG ATATCGTATT TTGTGTGCAC CTGTTGTGTG GGAGAATACA
```

FIGURE 3 CONTINUED

2641 ACCAGTCGCG ATCTGTACTT GCCTGTGCTG ACCAAATGGT ACAAATTCGG CCCTGACTAT
2701 GACACCAAGC GCCTGGATTC TGCGTTGGAT GGAGGGCAGA TGATTAAGAA CTATTCTGTG
2761 CCACAAAGCG ACTCTCCGAT ATTTGTGAGG GAAGGAGCTA TTCTCCCTAC CCGCTACACG
2821 TTGGACGGTT CGAACAAGTC AATGAACACG TACACAGACA AAGACCCGTT GGTGTTTGAG
2881 GTATTCCCTC TTGGAAACAA CCGTGCCGAC GGTATGTGTT ATCTTGATGA TGGCGGTATT
2941 ACTACAGATG CTGAGGACCA TGGCAAATTC TCTGTTATCA ATGTCGAAGC CTTACGGAAA
3001 GGTGTTACGA CGACGATCAA GTTTGCGTAT GACACTTATC AATACGTATT TGATGGTCCA
3061 TTCTACGTTC GAATCCGTAA TCTTACGACT GCATCAAAAA TTAACGTGTC TTCTGGAGCG
3121 GGTGAAGAGG ACATGACACC GACCTCTGCG AACTCGAGGG CAGCTTTGTT CAGTGATGGA
3181 GGTGTTGGAG AATACTGGGC TGACAATGAT ACGTCTTCTC TGTGGATGAA GTTGCCAAAC
3241 CTGGTTCTGC AAGACGCTGT GATTACCATT ACGTAG

FIGURE 9

α-GLUCAN LYASE CODING SEQUENCE
SEQUENCE TYPE: NUCLEIC ACID
MOLECULE TYPE: DNA (GENOMIC)
ORIGINAL SOURCE: FUNGUS
SEQUENCE LENGTH: 3201 BP
STRANDEDNESS: DOUBLE
SEQUENCE:

```
        10         20         30         40         50         60
ATGGCAGGAT TTTCTGATCC TCTCAACTTT TGCAAAGCAG AAGACTACTA CAGTGTTGCG
        70         80         90        100        110        120
CTAGACTGGA AGGGCCCTCA AAAAATCATT GGAGTAGACA CTACTCCTCC AAAGAGCACC
       130        140        150        160        170        180
AAGTTCCCCA AAAACTGGCA TGGAGTGAAC TTGAGATTCG ATGATGGGAC TTTAGGTGTG
       190        200        210        220        230        240
GTTCAGTTCA TTAGGCCGTG CGTTTGGAGG GTTAGATACG ACCCTGGTTT CAAGACCTCT
       250        260        270        280        290        300
GACGAGTATG GTGATGAGAA TACGAGGACA ATTGTGCAAG ATTATATGAG TACTCTGAGT
       310        320        330        340        350        360
AATAAATTGG ATACTTATAG AGGTCTTACG TGGGAAACCA AGTGTGAGGA TTCGGGAGAT
       370        380        390        400        410        420
TTCTTTACCT TCTCATCCAA GGTCACCGCC GTTGAAAAAT CCGAGCGGAC CCGCAACAAG
       430        440        450        460        470        480
GTCGGCGATG GCCTCAGAAT TCACCTATGG AAAAGCCCTT TCCGCATCCA AGTAGTGCGC
       490        500        510        520        530        540
ACCTTGACCC CTTTGAAGGA TCCTTACCCC ATTCCAAATG TAGCCGCAGC CGAAGCCCGT
       550        560        570        580        590        600
GTGTCCGACA AGGTCGTTTG GCAAACGTCT CCCAAGACAT TCAGAAAGAA CCTGCATCCG
       610        620        630        640        650        660
CAACACAAGA TGCTAAAGGA TACAGTTCTT GACATTGTCA AACCTGGACA TGGCGAGTAT
       670        680        690        700        710        720
GTGGGGTGGG GAGAGATGGG AGGTATCCAG TTTATGAAGG AGCCAACATT CATGAACTAT
       730        740        750        760        770        780
TTTAACTTCG ACAATATGCA ATACCAGCAA GTCTATGCCC AAGGTGCTCT CGATTCTCGC
       790        800        810        820        830        840
GAGCCACTGT ACCACTCGGA TCCCTTCTAT CTTGATGTGA ACTCCAACCC GGAGCACAAG
       850        860        870        880        890        900
AATATCACGG CAACCTTTAT CGATAACTAC TCTCAAATTG CCATCGACTT TGGAAAGACC
       910        920        930        940        950        960
AACTCAGGCT ACATCAAGCT GGGAACCAGG TATGGTGGTA TCGATTGTTA CGGTATCAGT
       970        980        990       1000       1010       1020
GCGGATACGG TCCCGGAAAT TGTACGACTT TATACAGGTC TTGTTGGACG TTCAAAGTTG
      1030       1040       1050       1060       1070       1080
AAGCCCAGAT ATATTCTCGG GGCCCATCAA GCCTGTTATG GATACCAACA GGAAAGTGAC
      1090       1100       1110       1120       1130       1140
TTGTATTCTG TGGTCCAGCA GTACCGTGAC TGTAAATTTC CACTTGACGG GATTCACGTC
      1150       1160       1170       1180       1190       1200
GATGTCGATG TTCAGGACGG CTTCAGAACT TTCACCACCA ACCCACACAC TTTCCCTAAC
      1210       1220       1230       1240       1250       1260
CCCAAAGAGA TGTTTACTAA CTTGAGGAAT AATGGAATCA AGTGCTCCAC CAATATCACT
      1270       1280       1290       1300       1310       1320
CCTGTTATCA GCATTAACAA CAGAGAGGGT GGATACAGTA CCCTCCTTGA GGGAGTTGAC
```

FIGURE 9 CONTINUED

```
      1330        1340        1350        1360        1370        1380
AAAAAATACT TTATCATGGA CGACAGATAT ACCGAGGGAA CAAGTGGGAA TGCGAAGGAT
      1390        1400        1410        1420        1430        1440
GTTCGGTACA TGTACTACGG TGGTGGTAAT AAGGTTGAGG TCGATCCTAA TGATGTTAAT
      1450        1460        1470        1480        1490        1500
GGTCGGCCAG ACTTTAAAGA CAACTATGAC TTCCCCGCGA ACTTCAACAG CAAACAATAC
      1510        1520        1530        1540        1550        1560
CCCTATCATG GTGGTGTGAG CTACGGTTAT GGGAACGGTA GTGCAGGTTT TTACCCGGAC
      1570        1580        1590        1600        1610        1620
CTCAACAGAA AGGAGGTTCG TATCTGGTGG GGAATGCAGT ACAAGTATCT CTTCGATATG
      1630        1640        1650        1660        1670        1680
GGACTGGAAT TTGTGTGGCA AGACATGACT ACCCCAGCAA TCCACACATC ATATGGAGAC
      1690        1700        1710        1720        1730        1740
ATGAAAGGGT TGCCCACCCG TCTACTCGTC ACCTCAgACT CCGTCACCAA TGCCTCTGAG
      1750        1760        1770        1780        1790        1800
AAAAAGCTCG CAATTGAAAC TTGGGCTCTC TACTCCTACA ATCTCCACAA AGCAACTTGG
      1810        1820        1830        1840        1850        1860
CATGGTCTTA GTCGTCTCGA ATCTCGTAAG AACAAACGAA ACTTCATCCT CGGGCGTGGA
      1870        1880        1890        1900        1910        1920
AGTTATGCCG GAGCCTATCG TTTTGCTGGT CTCTGGACTG GGGATAATGC AAGTAACTGG
      1930        1940        1950        1960        1970        1980
GAATTCTGGA AGATATCGGT CTCTCAAGTT CTTTCTCTGG GCCTCAATGG TGTGTGCATC
      1990        2000        2010        2020        2030        2040
GCGGGGTCTG ATACGGGTGG TTTTGAACCC TACCGTGATG CAAATGGGGT CGAGGAGAAA
      2050        2060        2070        2080        2090        2100
TACTGTAGCC CAGAGCTACT CATCAGGTGG TATACTGGTT CATTCCTCTT GCCGTGGCTC
      2110        2120        2130        2140        2150        2160
AGGAACCATT ATGTCAAAAA GGACAGGAAA TGGTTCCAGG AACCATACTC GTACCCCAAG
      2170        2180        2190        2200        2210        2220
CATCTTGAAA CCCATCCAGA ACTCGCAGAC CAAGCATGGC TCTATAAATC CGTTTTGGAG
      2230        2240        2250        2260        2270        2280
ATCTGTAGGT ACTATGTGGA GCTTAGATAC TCCCTCATCC AACTACTTTA CGACTGCATG
      2290        2300        2310        2320        2330        2340
TTTCAAAACG TaGTCGACGG TATGCCAATC ACCAGATCTA TGCTCTTGAC CGATACTGAG
      2350        2360        2370        2380        2390        2400
GATACCACCT TCTTCAACGA GAGCCAAAAG TTCCTCGACA ACCAATATAT GGCTGGTGAC
      2410        2420        2430        2440        2450        2460
GACATTCTTG TTGCACCCAT CCTCCACAGT CGCAAAGAAA TTCCAGGCGA AAACAGAGAT
      2470        2480        2490        2500        2510        2520
GTCTATCTCC CTCTTTACCA CACCTGGTAC CCCTCAAATT TGAGACCATG GGACGATCAA
      2530        2540        2550        2560        2570        2580
GGAGTCGCTT TGGGGAATCC TGTCGAAGGT GGTAGTGTCA TCAATTATAC TGCTAGGATT
      2590        2600        2610        2620        2630        2640
GTTGCACCCG AGGATTATAA TCTCTTCCAC AGCGTGGTAC CAGTCTACGT TAGAGAGGGT
      2650        2660        2670        2680        2690        2700
GCCATCATCC CGCAAATCGA AGTACGCCAA TGGACTGGCC AGGGGGGAGC CAACCGCATC
      2710        2720        2730        2740        2750        2760
AAGTTCAACA TCTACCCTGG AAAGGATAAG GAGTACTGTA CCTATCTTGA TGATGGTGTT
      2770        2780        2790        2800        2810        2820
AGCCGTGATA GTGCGCCGGA AGACCTCCCA CAGTACAAAG AGACCCACGA ACAGTCGAAG
      2830        2840        2850        2860        2870        2880
GTTGAAGGCG CGGAAATCGC AAAGCAGATT GGAAAGAAGA CGGGTTACAA CATCTCAGGA
      2890        2900        2910        2920        2930        2940
```

FIGURE 9 CONTINUED

```
ACCGACCCAG AAGCAAAGGG TTATCACCGC AAAGTTGCTG TCACACAAAC GTCAAAAGAC
      2950       2960       2970       2980       2990       3000
AAGACGCGTA CTGTCACTAT TGAGCCAAAA CACAATGGAT ACGACCCTTC CAAAGAGGTG
      3010       3020       3030       3040       3050       3060
GGTGATTATT ATACCATCAT TCTTTGGTAC GCACCAGGTT TCGATGGCAG CATCGTCGAT
      3070       3080       3090       3100       3110       3120
GTGAGCAAGA CGACTGTGAA TGTTGAGGGT GGGGTGGAGC ACCAAGTTTA TAAGAACTCC
      3130       3140       3150       3160       3170       3180
GATTTACATA CGGTTGTTAT CGACGTGAAG GAGGTGATCG GTACCACAAA GAGCGTCAAG
      3190       3200
ATCACATGTA CTGCCGCTTA A
```

FIGURE 10

α-GLUCAN LYASE CODING SEQUENCE
SEQUENCE TYPE: NUCLEIC ACID
MOLECULE TYPE: DNA (GENOMIC)
ORIGINAL SOURCE: FUNGUS
SEQUENCE LENGTH: 3213 BP
STRANDEDNESS: DOUBLE
SEQUENCE:

```
        10          20          30          40          50          60
ATGGCAGGAT TATCCGACCC TCTCAATTTC TGCAAAGCAG AGGACTACTA CGCTGCTGCC
        70          80          90         100         110         120
AAAGGCTGGA GTGGCCCTCA GAAGATCATT CGCTATGACC AGACCCCTCC TCAGGGTACA
       130         140         150         160         170         180
AAAGATCCGA AAAGCTGGCA TGCGGTAAAC CTTCCTTTCG ATGACGGGAC TATGTGTGTA
       190         200         210         220         230         240
GTGCAATTCG TCAGACCCTG TGTTTGGAGG GTTAGATATG ACCCCAGTGT CAAGACTTCT
       250         260         270         280         290         300
GATGAGTACG GCGATGAGAA TACGAGGACT ATTGTACAAG ACTACATGAC TACTCTGGTT
       310         320         330         340         350         360
GGAAACTTGG ACATTTTCAG AGGTCTTACG TGGGTTTCTA CGTTGGAGGA TTCGGGCGAG
       370         380         390         400         410         420
TACTACACCT TCAAGTCCGA AGTCACTGCC GTGGACGAAA CCGAACGGAC TCGAAACAAG
       430         440         450         460         470         480
GTCGGCGACG GCCTCAAGAT TTACCTATGG AAAAATCCCT TTCGCATCCA GGTAGTGCGT
       490         500         510         520         530         540
CTCTTGACCC CCCTGGTGGA CCCTTTCCCC ATTCCCAACG TAGCCAATGC CACAGCCCGT
       550         560         570         580         590         600
GTGGCCGACA AGGTTGTTTG GCAGACGTCC CCGAAGACGT TCAGGAAAAA CTTGCATCCG
       610         620         630         640         650         660
CAGCATAAGA TGTTGAAGGA TACAGTTCTT GATATTATCA AGCCGGGGCA CGGAGAGTAT
       670         680         690         700         710         720
GTGGGTTGGG GAGAGATGGG AGGCATCGAG TTTATGAAGG AGCCAACATT CATGAATTAT
       730         740         750         760         770         780
TTCAACTTTG ACAATATGCA ATATCAGCAG GTCTATGCAC AAGGCGCTCT TGATAGTCGT
       790         800         810         820         830         840
GAGCCGTTGT ATCACTCTGA TCCCTTCTAT CTCGACGTGA ACTCCAACCC AGAGCACAAG
       850         860         870         880         890         900
AACATTACGG CAACCTTTAT CGATAACTAC TCTCAGATTG CCATCGACTT TGGGAAGACC
       910         920         930         940         950         960
AACTCAGGCT ACATCAAGCT GGGTACCAGG TATGGCGGTA TCGATTGTTA CGGTATCAGC
       970         980         990        1000        1010        1020
GCGGATACGG TCCCGGAGAT TGTGCGACTT TATACTGGAC TTGTTGGGCG TTCGAAGTTG
      1030        1040        1050        1060        1070        1080
AAGCCCAGGT ATATTCTCGG AGCCCACCAA GCTTGTTATG GATACCAGCA GGAAAGTGAC
      1090        1100        1110        1120        1130        1140
TTGCATGCTG TTGTTCAGCA GTACCGTGAC ACCAAGTTTC CGCTTGATGG GTTGCATGTC
      1150        1160        1170        1180        1190        1200
GATGTCGACT TTCAGGACAA TTTCAGAACG TTTACCACTA ACCCGATTAC GTTCCCTAAT
      1210        1220        1230        1240        1250        1260
CCCAAAGAAA TGTTTACCAA TCTAAGGAAC AATGGAATCA AGTGTTCCAC CAACATCACC
      1270        1280        1290        1300        1310        1320
```

FIGURE 10 CONTINUED

CCTGTTATCA GTATCAGAGA TCGCCCGAAT GGGTACAGTA CCCTCAATGA GGGATATGAT
1330 1340 1350 1360 1370 1380
AAAAAGTACT TCATCATGGA TGACAGATAT ACCGAGGGGA CAAGTGGGGA CCCGCAAAAT
1390 1400 1410 1420 1430 1440
GTTCGATACT CTTTTTACGG CGGTGGGAAC CCGGTTGAGG TTAACCCTAA TGATGTTTGG
1450 1460 1470 1480 1490 1500
GCTCGGCCAG ACTTTGGAGA CAATTATGAC TTCCCTACGA ACTTCAACTG CAAAGACTAC
1510 1520 1530 1540 1550 1560
CCCTATCATG GTGGTGTGAG TTACGGATAT GGGAATGGCA CTCCAGGTTA CTACCCTGAC
1570 1580 1590 1600 1610 1620
CTTAACAGAG AGGAGGTTCG TATCTGGTGG GGATTGCAGT ACGAGTATCT CTTCAATATG
1630 1640 1650 1660 1670 1680
GGACTAGAGT TTGTATGGCA AGATATGACA ACCCCAGCGA TCCATTCATC ATATGGAGAC
1690 1700 1710 1720 1730 1740
ATGAAAGGGT TGCCCACCCG TCTGCTCGTC ACCGCCGACT CAGTTACCAA TGCCTCTGAG
1750 1760 1770 1780 1790 1800
AAAAAGCTCG CAATTGAAAG TTGGGCTCTT TACTCCTACA ACCTCCATAA AGCAACCTTC
1810 1820 1830 1840 1850 1860
CACGGTCTTG GTCGTCTTGA GTCTCGTAAG AACAAACGTA ACTTCATCCT CGGACGTGGT
1870 1880 1890 1900 1910 1920
AGTTACGCCG GTGCCTATCG TTTTGCTGGT CTCTGGACTG GAGATAACGC AAGTACGTGG
1930 1940 1950 1960 1970 1980
GAATTCTGGA AGATTTCGGT CTCCCAAGTT CTTTCTCTAG GTCTCAATGG TGTGTGTATA
1990 2000 2010 2020 2030 2040
GCGGGGTCTG ATACGGGTGG TTTTGAGCCC GCACGTACTG AGATTGGGGA GGAGAAATAT
2050 2060 2070 2080 2090 2100
TGCAGTCCGG AGCTACTCAT CAGGTGGTAT ACTGGATCAT TCCTTTTGCC ATGGCTTAGA
2110 2120 2130 2140 2150 2160
AACCACTACG TCAAGAAGGA CAGGAAATGG TTCCAGGAAC CATACGCGTA CCCCAAGCAT
2170 2180 2190 2200 2210 2220
CTTGAAACCC ATCCAGAGCT CGCAGATCAA GCATGGCTTT ACAAATCTGT TCTAGAAATT
2230 2240 2250 2260 2270 2280
TGCAGATACT GGGTAGAGCT AAGATATTCC CTCATCCAGC TCCTTTACGA CTGCATGTTC
2290 2300 2310 2320 2330 2340
CAAAACGTGG TCGATGGTAT GCCACTTGCC AGATCTATGC TCTTGACCGA TACTGAGGAT
2350 2360 2370 2380 2390 2400
ACGACCTTCT TCAATGAGAG CCAAAAGTTC CTCGATAACC AATATATGGC TGGTGACGAC
2410 2420 2430 2440 2450 2460
ATCCTTGTAG CACCCATCCT CCACAGCCGT AACGAGGTTC CGGGAGAGAA CAGAGATGTC
2470 2480 2490 2500 2510 2520
TATCTCCCTC TATTCCACAC CTGGTACCCC TCAAACTTGA GACCGTGGGA CGATCAGGGA
2530 2540 2550 2560 2570 2580
GTCGCTTTAG GGAATCCTGT CGAAGGTGGC AGCGTTATCA ACTACACTGC CAGGATTGTT
2590 2600 2610 2620 2630 2640
GCCCCAGAGG ATTATAATCT CTTCCACAAC GTGGTGCCGG TCTACATCAG AGAGGGTGCC
2650 2660 2670 2680 2690 2700
ATCATTCCGC AAATTCAGGT ACGCCAGTGG ATTGGCGAAG GAGGGCCTAA TCCCATCAAG
2710 2720 2730 2740 2750 2760
TTCAATATCT ACCCTGGAAA GGACAAGGAG TATGTGACGT ACCTTGATGA TGGTGTTAGC
2770 2780 2790 2800 2810 2820
CGCGATAGTG CACCAGATGA CCTCCCGCAG TACCGCGAGG CCTATGAGCA AGCGAAGGTC
2830 2840 2850 2860 2870 2880

FIGURE 10 CONTINUED

```
GAAGGCAAAG ACGTCCAGAA GCAACTTGCG GTCATTCAAG GGAATAAGAC TAATGACTTC
      2890        2900        2910        2920        2930        2940
TCCGCCTCCG GGATTGATAA GGAGGCAAAG GGTTATCACC GCAAAGTTTC TATCAAACAG
      2950        2960        2970        2980        2990        3000
GAGTCAAAAG ACAAGACCCG TACTGTCACC ATTGAGCCAA AACACAACGG ATACGACCCC
      3010        3020        3030        3040        3050        3060
TCTAAGGAAG TTGGTAATTA TTATACCATC ATTCTTTGGT ACGCACCGGG CTTTGACGGC
      3070        3080        3090        3100        3110        3120
AGCATCGTCG ATGTGAGCCA GGCGACCGTG AACATCGAGG GCGGGGTGGA ATGCGAAATT
      3130        3140        3150        3160        3170        3180
TTCAAGAACA CCGGCTTGCA TACGGTTGTA GTCAACGTGA AAGAGGTGAT CGGTACCACA
      3190        3200        3210
AAGTCCGTCA AGATCACTTG CACTACCGCT TAG
```

FIG. 11

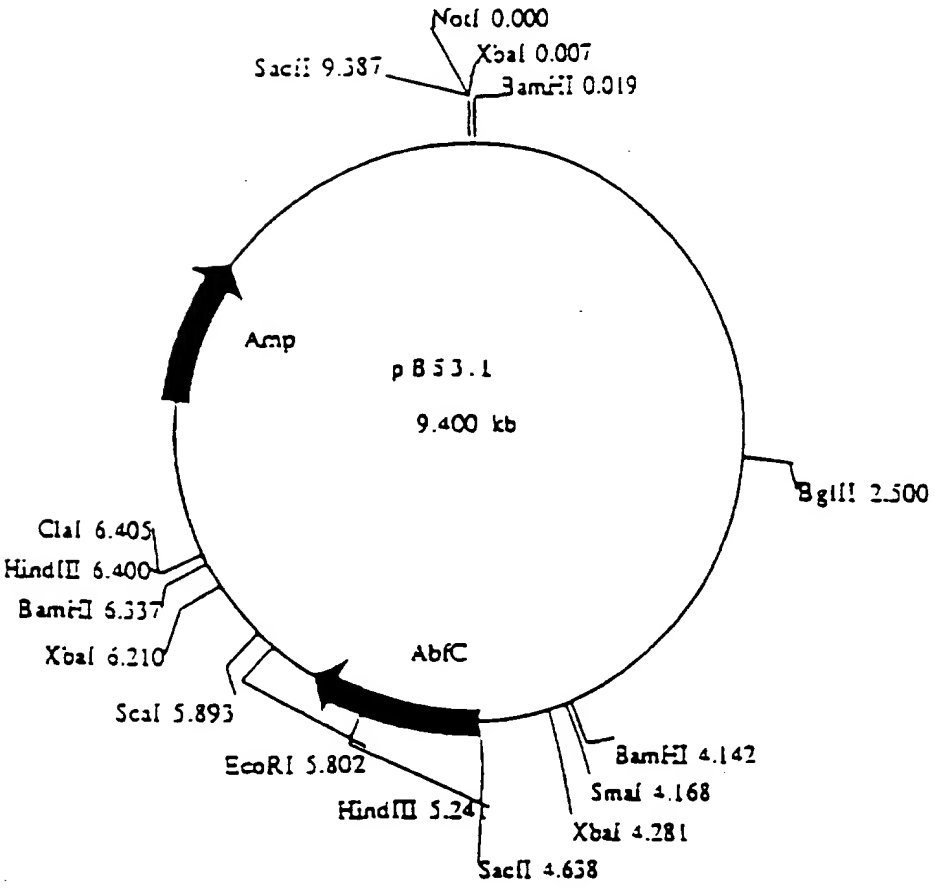

NotI 0.000
XbaI 0.007
BamHI 0.019
SacII 9.387
Amp
pB53.1
9.400 kb
BglII 2.500
ClaI 6.405
HindIII 6.400
BamHI 6.337
XbaI 6.210
ScaI 5.893
AbfC
EcoRI 5.802
HindIII 5.241
SacII 4.638
BamHI 4.142
SmaI 4.168
XbaI 4.281

FIG. 12

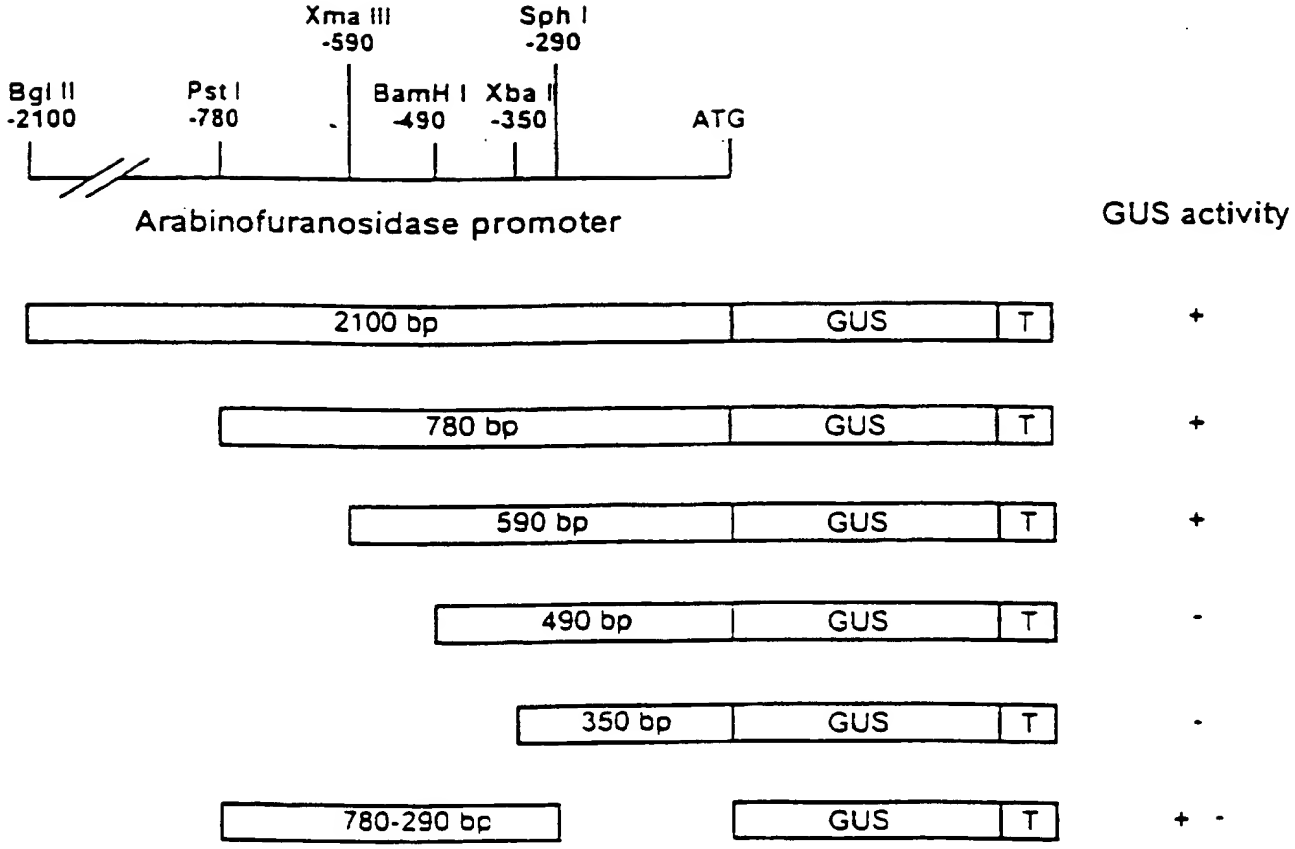

Xma III
-590
Sph I
-290
Bgl II
-2100
Pst I
-780
BamH I
-490
Xba I
-350
ATG
Arabinofuranosidase promoter
GUS activity
2100 bp
GUS
T
+
780 bp
GUS
T
+
590 bp
GUS
T
+
490 bp
GUS
T
-
350 bp
GUS
T
-
780-290 bp
GUS
T
+ -

FIG. 13

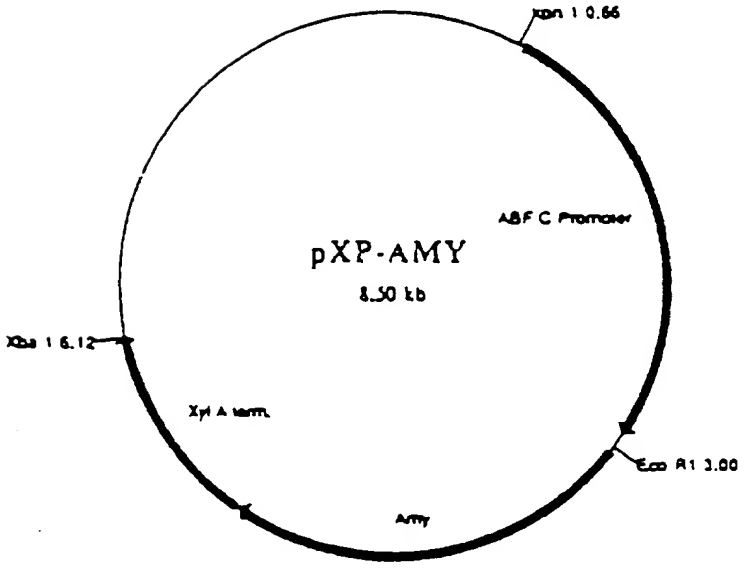
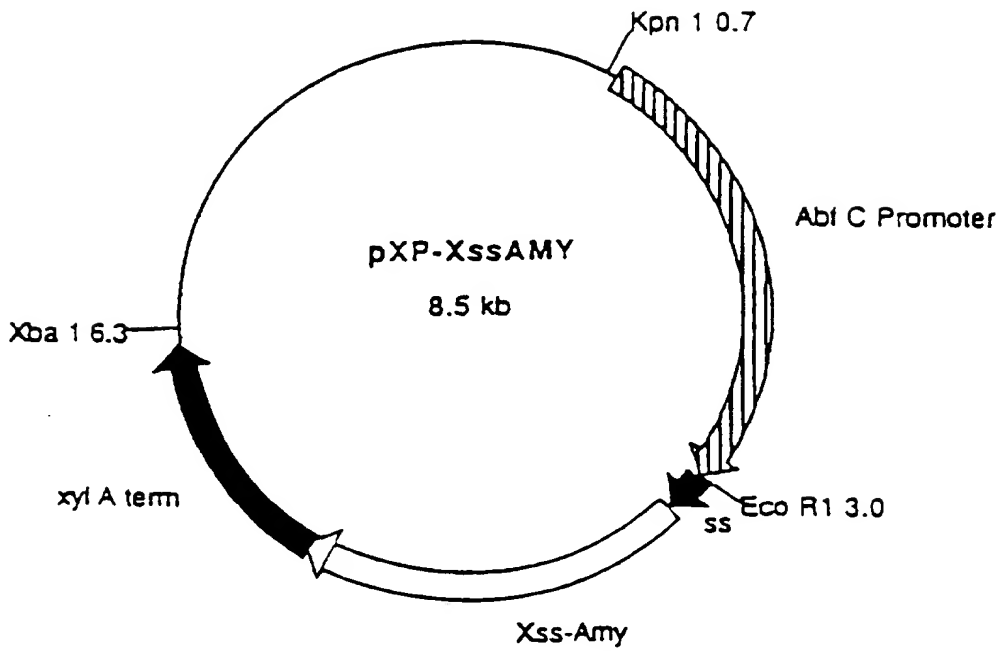

Xba I 0.66
ABF C Promoter
pXP-AMY
8.50 kb
Xba I 6.12
Xyl A term.
Eco R1 3.00
Amy

FIG. 14


Kpn 1 0.7
Abf C Promoter
pXP-XssAMY
8.5 kb
Xba 1 6.3
xyl A term
Eco R1 3.0
ss
Xss-Amy

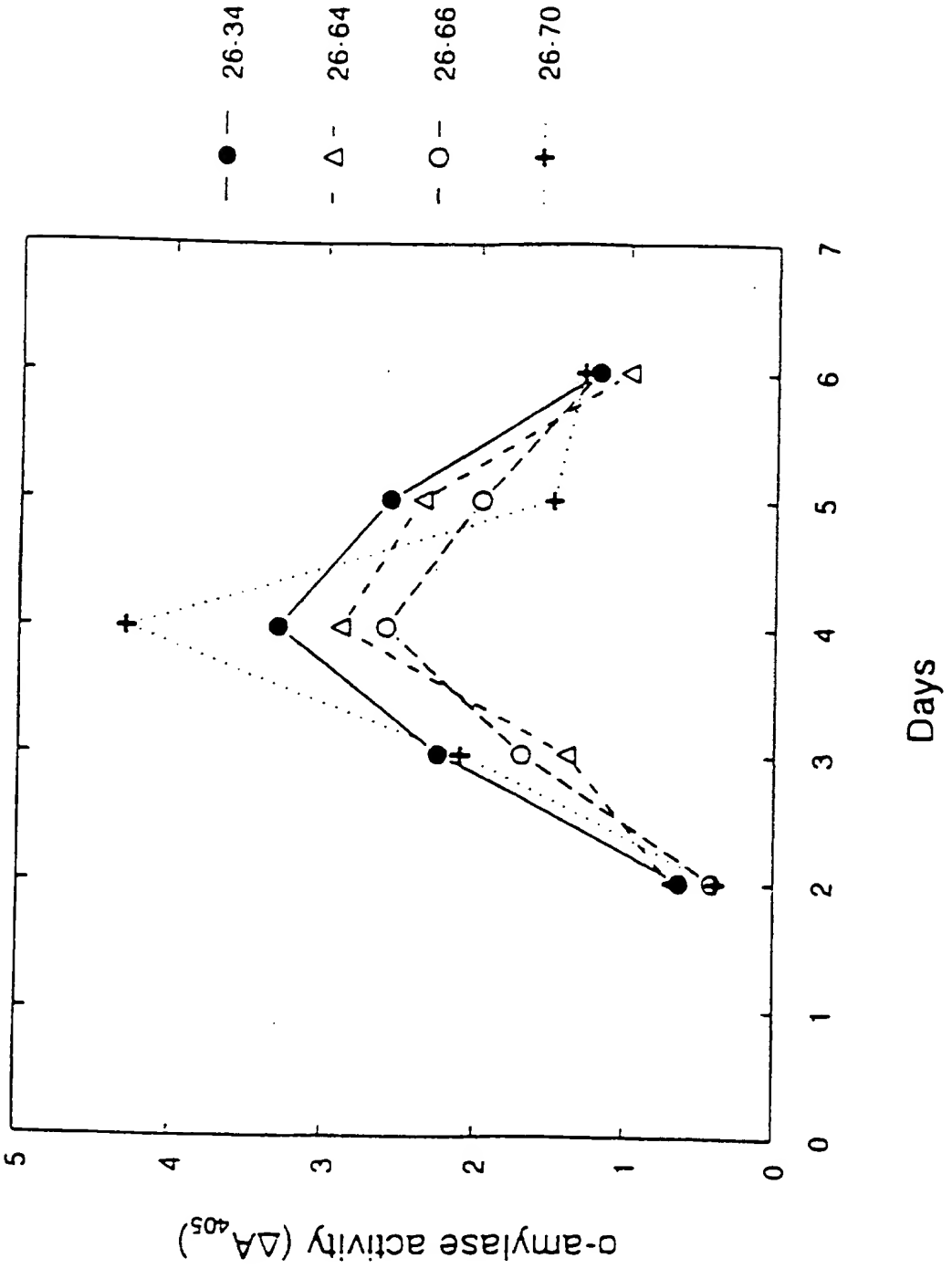

26·34
26·64
26·66
26·70
α-amylase activity (ΔA_{405})
Days
0
1
2
3
4
5
6
7

FIG. 15

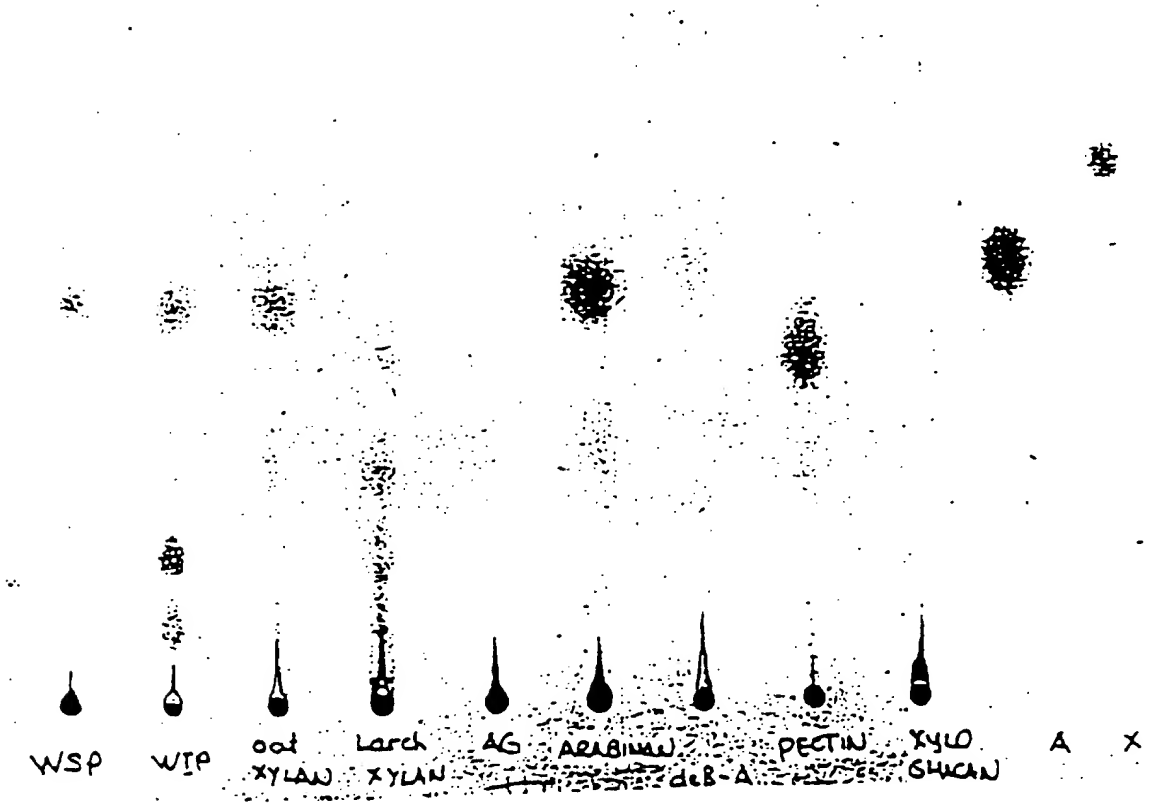

WSP
WIP
oat XYLAN
larch XYLAN
AG
ARABINAN
deB-A
PECTIN
XYLO GLUCAN
A
X

FIG. 16

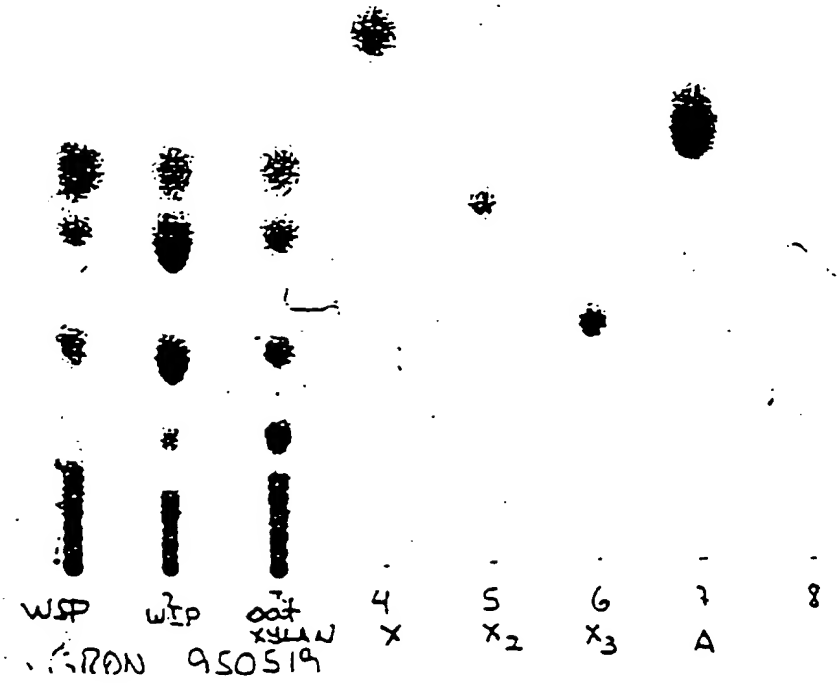
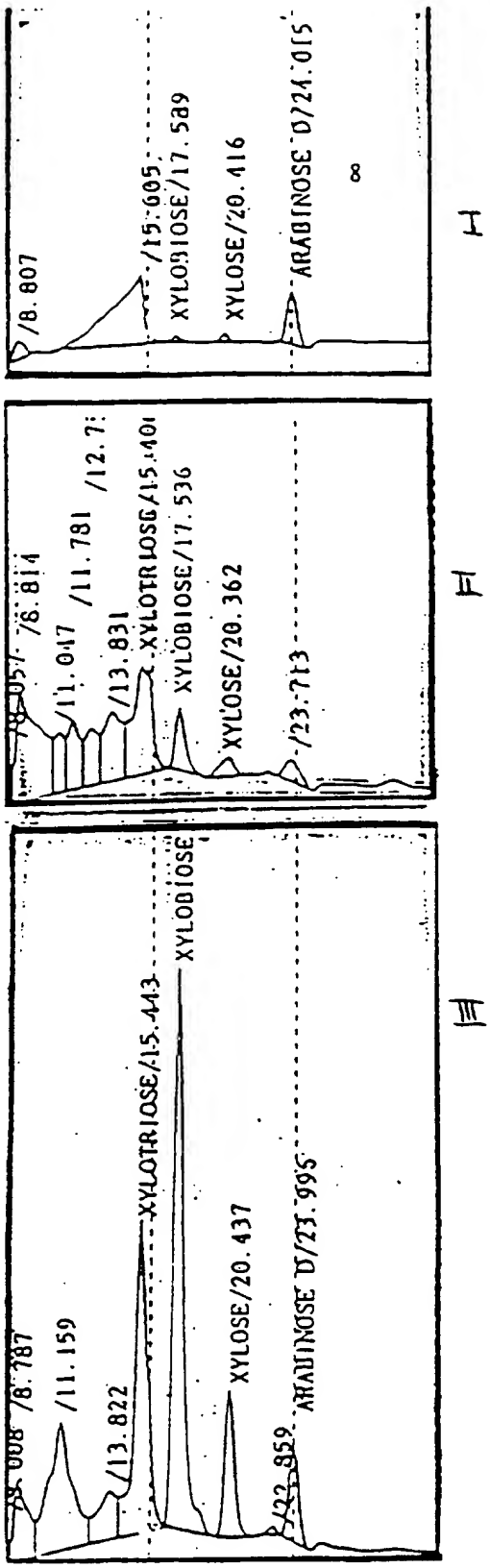


FIG. 17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/15.605
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XYLOSE/20.416
ARABINOSE D/24.015
b
/8.814
/11.047
/11.781
/13.831
XYLOTRIOSE/15.401
XYLOBIOSE/17.536
XYLOSE/20.362
/23.713
c
/8.787
/11.159
/13.822
XYLOTRIOSE/15.443
XYLOBIOSE
XYLOSE/20.437
/22.859
ARABINOSE D/23.995

FIG. 18

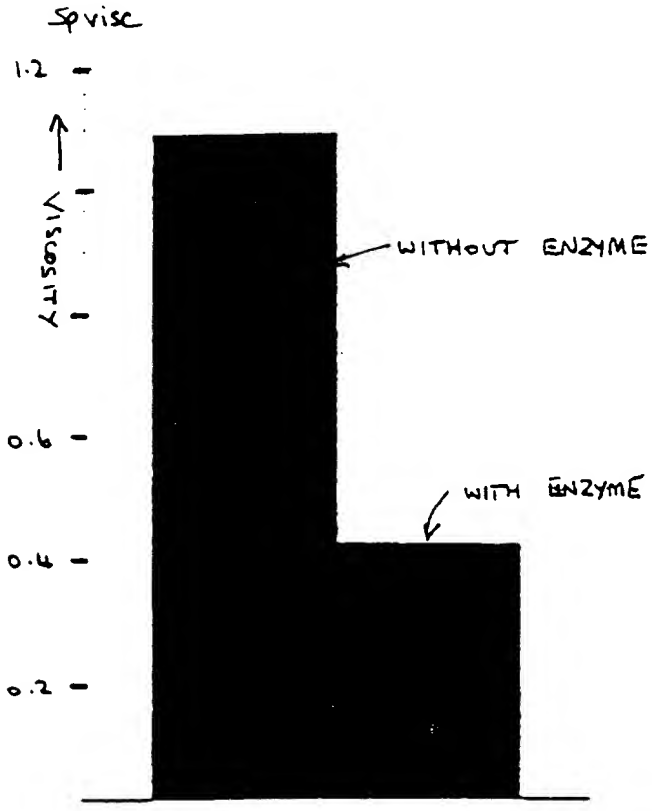


FIG. 19

Fig 20

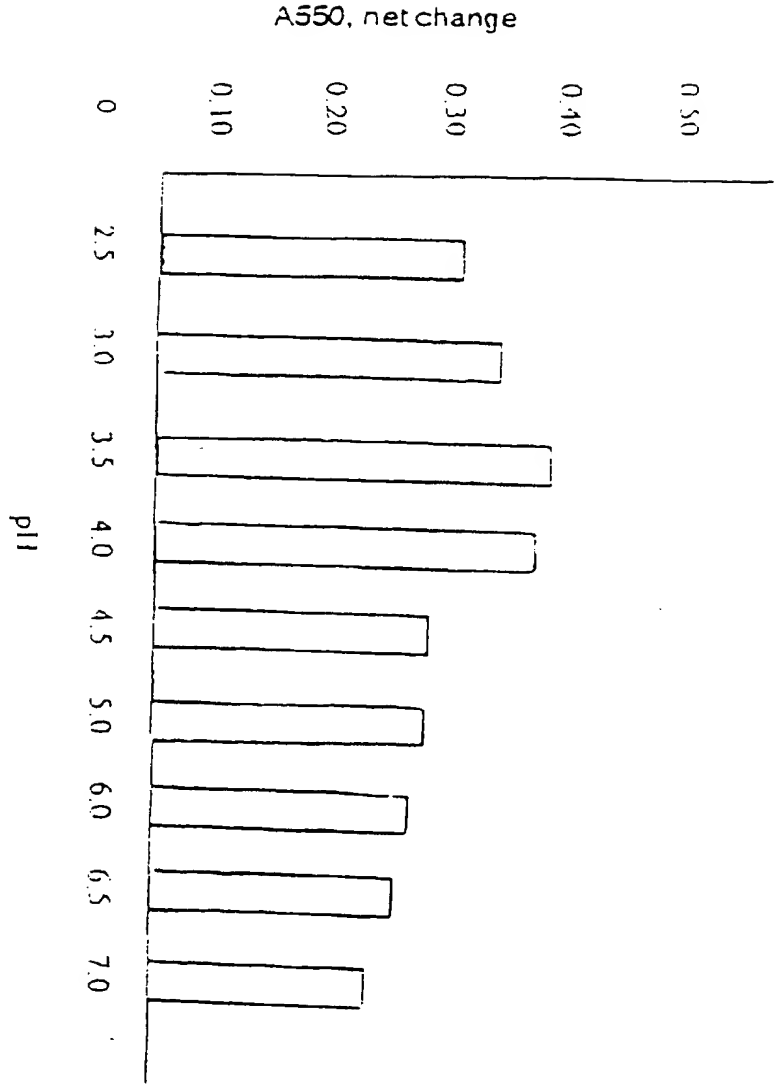
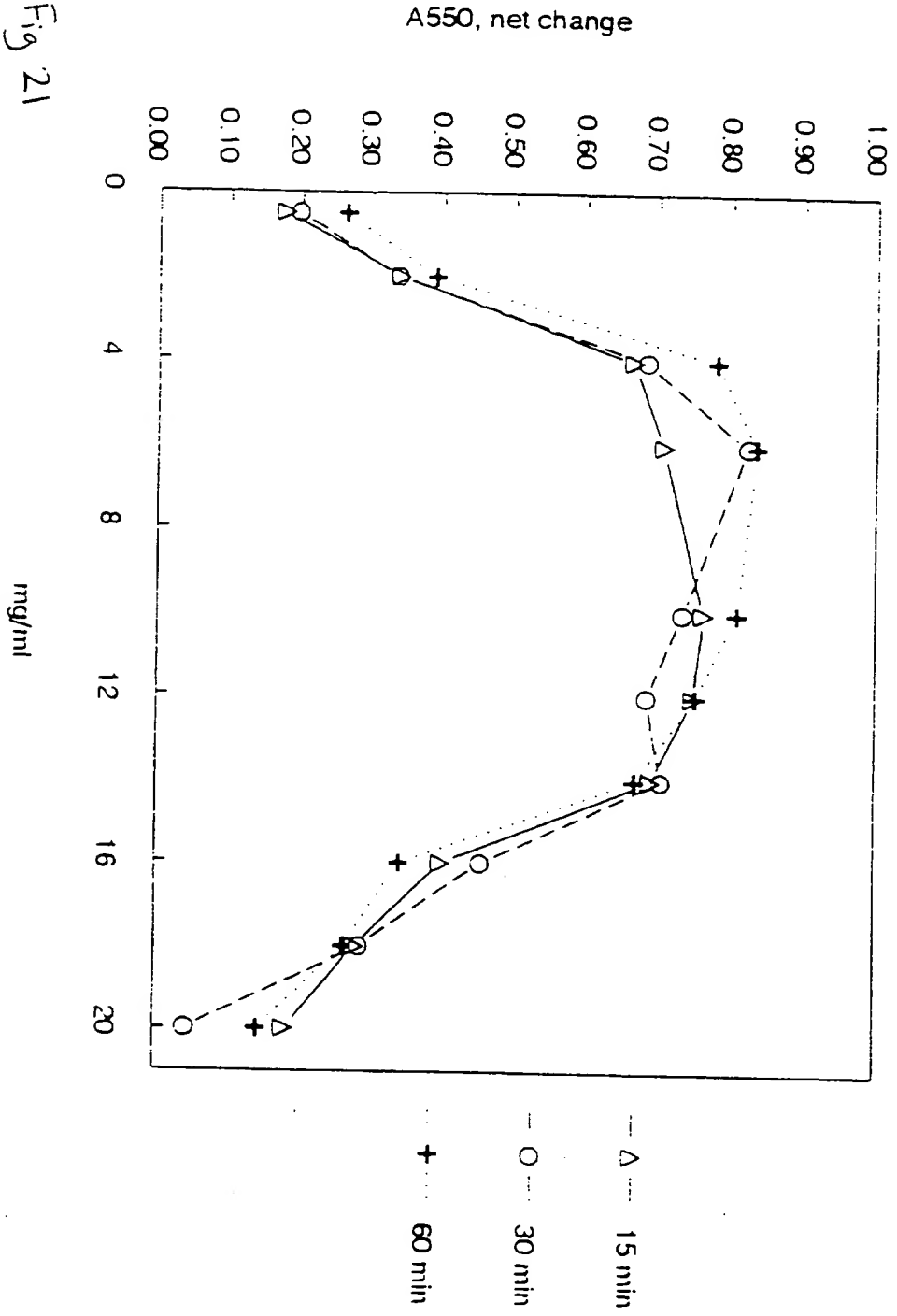


Fig 21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0.20
0.30
0.40
0.50
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0.90
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0
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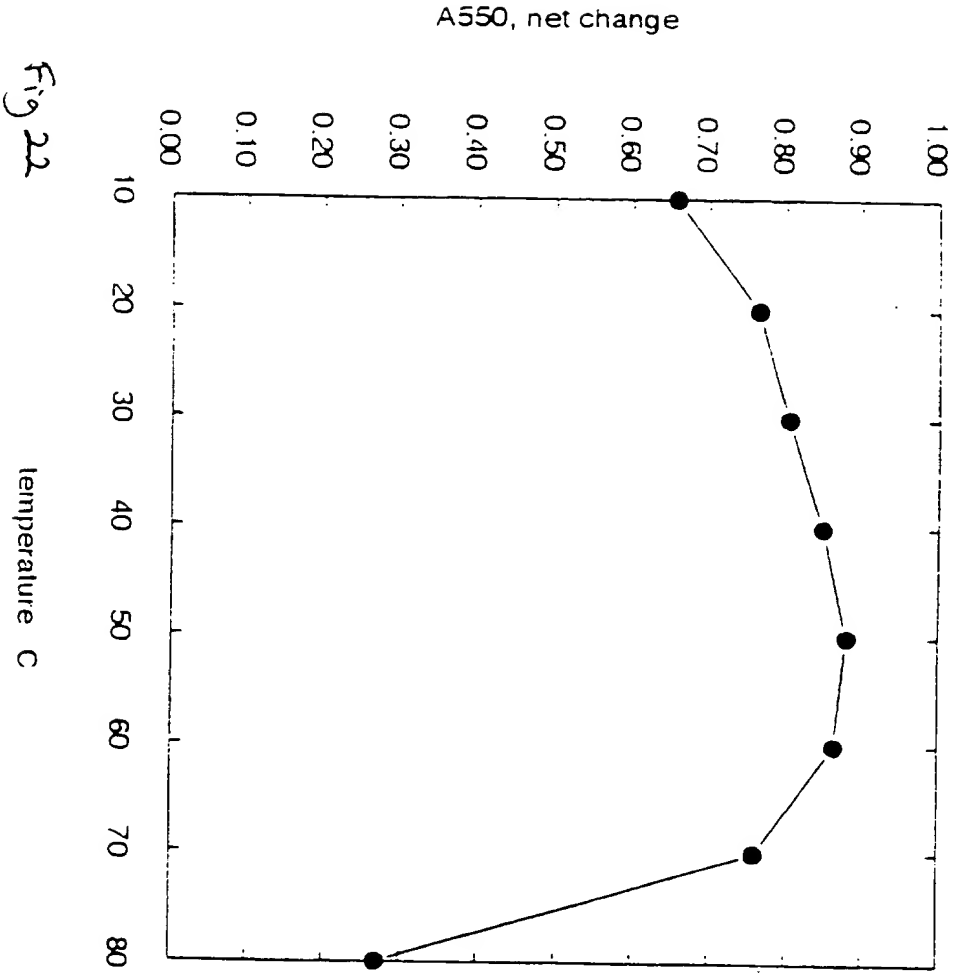
8
12
16
20
mg/ml
15 min
30 min
60 min

Fig 22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0.60
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0.80
0.90
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10
20
30
40
50
60
70
80
temperature C

INTERNATIONAL SEARCH REPORT

tional Application No
PCT/EP 96/01009

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/56 C12N15/80 C12N1/15 C12P19/04 A01H5/00
A61K38/47 A23L1/29

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12P A01H A61K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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P,X	WO,A,96 06935 (GIST BROCADES BV ;WOUW MONIQUE JOSINA ANDREA V D (NL); OOIJEN ALBE) 7 March 1996 see the whole document --- -/--	1-19, 21-25, 29-31

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
19 July 1996	29. 07. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Maddox, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

International Application No
PCT/EP 96/01009

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